ABSTRACT

KIRSTIN BRUST. Studies in Oxidative Chemistry: Oxidation of Benz[a]anthracene using a Synthetic Iron Porphyrin Catalyst to Model Cytochrome P-450. (Under the direction of DR. AVRAM GOLD)

Oxidations of the PAH benz[a]anthracene were carried out using m-chloroperoxybenzoic acid as monooxygen donor and the 2,6-chlorotetraphenyl-substituted iron porphyrin as catalyst. These reactions were performed in two different modes. The first used the oxoferryl porphyrin cation radical as direct oxidant, essentially allowing only one turnover of catalyst, whereas the second used the highvalent complex in a multi-turnover, catalytic mode. Phenoxathiin hexachloroantimonate was used as a model oneelectron oxidant to compare its product profile to the other modes. Identical products (or substitution at the same carbon) in the other modes would provide support for a oneelectron pathway. 180-labelled mCPBA was used in oxidations to provide direct evidence for involvement of the peroxyacid as the monooxygen donor, and 180 incorporation in the oxidized PAH was observed. Analysis of the product profiles in the three different oxidations supports one-electron oxidation as the major pathway operating. The products of the porphinatoiron-mediated oxidation are also postulated to depend on the relative amounts of substrate and available catalyst present during the oxidation.

ACKNOWLEDGEMENTS

I would like to thank Dr. Avram Gold for giving me the opportunity to work in his laboratory on this project, and for all his help and guidance throughout. I also greatly appreciate the time spent by both Dr. Jayaraj and Dr. Sangaiah in assisting me with procedures and explaining the chemistry involved. I am also grateful to Dr. Ball and Dr. Aitken for taking the time to read this document and offering their comments and suggestions.

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CHAPTER I

INTRODUCTION

The metabolism of polycyclic aromatic hydrocarbons (PAH) is mediated by a class of heme proteins known as cytochromes P-450, and activation by these enzymes is thought to be requisite for PAH to exert carcinogenic or mutagenic effects. The P-450 complex functions as a monooxygenase and carries out oxidative reactions on a wide variety of xenobiotics. Hydroxylation by cytochrome P-450 involves the uptake of two electrons from NADPH with the concomitant reduction of one atom of O_2 to water; the other oxygen atom is incorporated into the substrate (White and Coon 1980). While this conversion usually produces a more excretable metabolite, it can also result in the formation of reactive intermediates that are either toxic or carcinogenic.

In mammalian liver homogenates, initial insertion of an oxygen across the double bond of an aromatic system to form an arene oxide has been shown to be a key step in the activation of PAH (Jerina and Daly, 1974). The arene oxide can undergo one of two enzyme-catalyzed reactions. Hydration by epoxide hydrolase results in a *trans*- dihydrodiol, or a glutathione moiety can be added by glutathione-S-epoxide transferase. The epoxide is also subject to non-enzymatic isomerization to a phenol (Dünges, 1973). This rearrangement, which can result in retention of substituents at the oxidized position via a 1,2-migration, is termed the NIH shift (Jerina and Daly, 1974).

Cytochrome P-450 can also carry out oxidations of hydrocarbons in the presence of iodosyl benzene and peroxyacid monooxygen donors (Hrycay and O'Brien, 1974). Since O2 or reducing cofactors are not involved, cytochrome P-450 is functioning in these reactions as a peroxidase, an enzyme that utilizes H2O2 as the ultimate electron acceptor. Horseradish peroxidase (HRP) and its intermediates have been extensively characterized. During catalysis, HRP is initially oxidized by two electrons to a green species known as compound I. It has been postulated that one of the two oxidation equivalents in compound I is stored as low spin ferryl iron (Fe(III)-->Fe(IV)), while the other oxidation equivalent is stored as a porphyrin centered m-cation radical. The techniques of Mössbauer spectroscopy and EPR lend support to this theory. From Mössbauer studies it was suggested that the iron is in the Fe(IV) state (Moss, Ehrenberg and Bearden, 1969), while EPR data have been consistent with the presence of a free radical relaxed by a nearby iron (Aasa, Vanngård, and Dunford, 1975). Based on the peroxidase activity of cytochrome P-450, its active oxygen species is believed to be similar on a structural

level to the high valent intermediate formed during the catalytic cycle of HRP.

Due to this presumed similarity, there have been numerous studies performed using synthetic catalysts with monooxygen donors to model the active center of cytochrome P-450. Among the many studies, only a few have rigorously demonstrated the presence of a compound I analogue (Gold et al., 1988; Groves et al., 1981, 1986). While substituted porphyrins in biomimetic oxidations do not approach the complexity of an enzymatic protein, insight into the mechanism of oxidation can be gained. Therefore, model oxidations on selected PAH were carried out employing porphyrin complexes shown to form observable compound I analogues when a peroxyacid is supplied as the monooxygen source.

The synthetic catalyst employed was 2,6-dichlorophenylsubstituted iron porphyrin. Formation of the compound I analogue [TPP(2,6-Cl)FeO]+ (Fig. 1) has been confirmed by spectroscopic and resonance studies. This porphyrin possesses strongly electron withdrawing ligands on its periphery and its ferryl-porphyrin radical spin are strongly parallel coupled. Presence of chlorine on the phenyl rings should render the cation radical intermediate more strongly oxidizing. The specific ramifications of the spin coupling characteristics pertaining to reactivity are still not clear, but it is important to note that all known compound I derivatives of enzymes possess weak or anti-parallel



Fig. 1. Structure of 2,6-dichlorophenylsubstituted iron porphyrin. TPP(2,6-Cl)Fe(III)



Fig. 2. Benz[a]anthracene, an alternant PAH used in the oxidations.

coupling. In the future, oxidation experiments can be carried out using a high-valent complex (as the catalyst) having antiferromagnetic coupling, to see what effect this has on reaction pathway and product profiles. Benz[a]anthracene, an alternant PAH containing four fused benzene rings (Fig. 2), was selected as substrate for the chemical oxidations.

One objective of this study was to determine whether the reaction pathway proceeds via direct electrophilic attack by the oxo oxygen on the PAH periphery, or initial one-electron charge transfer, in which the PAH is oxidized to a cation radical. To help clarify this point the oxidations were performed in two different ways, and the major products that resulted were compared. The first employed the oxoferryl porphyrin cation radical as a direct oxidant, generating the green compound I analogue, to which the substrate was subsequently added. The second set of oxidations was carried out in a truly catalytic mode using a small concentration of porphyrin catalyst and excess substrate, to which was added an approximate 100-fold excess of m-chloroperoxybenzoic acid (mCPBA). This allows the porphryin to "turn over", and simulates the enzymatic reaction which cytochrome P-450 catalyzes in a biological system. Similar product profiles in the two modes would enable one to justify use of multi-turnover conditions to model the peroxidase activity of cytochrome P-450.

An additional set of oxidations was carried out using phenoxythiin hexachloroantimonate as the oxidant. This compound is an effective electron acceptor, and is known to carry out one-electron transfer exclusively. Data generated from these studies will lend insight into the likelihood of one-electron transfer in the other modes if identical products are obtained.

The regioselectivity of oxidation is determined by steric constraints that the iron(V)-oxo complex encounters when it reacts with the PAH, as well as the charge distribution properties of the aromatic system (see Fig. 3). For this reason, distinguishing between these two pathways and proving which one is operating is not always straightforward. We wished to compare the product distributions obtained under both sets of conditions using the porphyrin catalyst with the product distribution obtained by the known one-electron oxidant as a means of identifying the pathways involved.

Peripheral attack by oxo oxygen will give initial epoxidations at the most localized aromatic bonds:

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which could lead to the following phenols by rearrangement during workup:

8-OH B(a)A



5-OH B(a)A



11-OH B(a)A



6-OH B(a)A



One electron oxidation would result in charge distributed at the meso carbon:



leading to products of oxidation at C7 or C7 and C12.

Fig. 3. Expected oxidation products.

CHAPTER II LITERATURE SEARCH

Metabolic Activation of PAH

Polycyclic aromatic hydrocarbons are compounds containing carbon and hydrogen with fused benzene rings in linear, angular and cluster arrangements. They are universal products of the combustion of organic matter, and their occurence in the environment is due to both natural and anthropogenic sources (Blumer, 1976).

The enzymes responsible for bioactivation of PAH and other xenobiotics are localized primarily in the endoplasmic reticulum of hepatocytes. Of these proteins, the most important are the oxidative enzymes in the cytochrome P-450 system, also termed the mixed-function oxygenase (MFO) system. Other enzymes involved in mammalian PAH metabolism include epoxide reductase, epoxide hydrolase, glutathione transferase, UDP-glucuronyl transferase, and sulfotransferase, and these pathways are shown in Fig. 4.

In general, the activity of these enzymes renders the substrate more polar and facilitates excretion from the cell and body. In some cases, however, the intermediates become electrophilic (electron deficient) and bind to nucleophilic (electron rich) sites on nucleic acids and proteins, and



Fig. 4. Major pathways involved in the mammalian metabolism of polycyclic aromatic hydrocarbons. (from Cerniglia 1984, p.34) certain nucleic acid adducts are considered to have the potential to initiate malignant transformation. Thus, the same enzymes that serve as detoxifiers can also act to increase the toxicity of certain xenobiotics.

Most PAH are not reactive or carcinogenic as the parent compound and require activation to be converted to a species that is biologically active with no further metabolic transformation, called an ultimate carcinogen. One oxidative pathway of PAH and olefins involves addition of oxygen across the C-C double bond. This reaction is carried out by a monooxygenase, and results in the formation of an epoxide. Important indirect evidence for an epoxide as an intermediate is the demonstration of an NIH shift (Fig. 5), that often accompanies rearrangement of an arene oxide to a phenol. The NIH shift involves the intramolecular migration of ring substituents, from the position where the oxygen is introduced to a neighboring position on the ring. The strained three-membered ring of the epoxide intermediate results in a highly reactive species, and upon opening to an electrophilic carbonium ion it seeks nucleophilic sites within the cell.

The extent of binding of this electrophile will depend on the rate of formation of arene oxides and the rates of hydration (both enzymatic and non-enzymatic) to dihydrodiols, conjugation with glutathione, and isomerization to phenols (Fig. 6). Where oxidation products are concerned, the relative proportions of different phenols



Fig. 5. The NIH shift. This migration and retention of substituents occurs during the monooxygenase-catalyzed formation of phenols.



Fig. 6. Three possible metabolic fates of an arene oxide: 1) Isomerization to a phenol 2) Hydration to a dihydrodiol 3) Conjugation with glutathione.

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formed during isomerization usually depend on the stability of the corresponding carbonium ion intermediate (Jerina et al., 1971).

Metabolism of Benzlalanthracene

The bacterium Beijerinckia sp. strain B-836 metabolizes benz[a]anthracene to four cis-dihydrodiols, formed by the incorporation of both atoms of molecular oxygen into the aromatic nucleus, and carried out by a dioxygenase. Fungi, in contrast, oxidize PAHs via cytochrome P-450 monooxygenase and epoxide hydrolase-catalyzed reactions to transdihydrodiols. The fungus C. elegans was shown to oxidize B[a]A predominantly to trans-8,9-dihydroxy-8,9dihydrobenz[a]anthracene (Cerniglia et al., 1980).

Benz[a]anthracene is metabolized by mammalian microsomal preparations and by a purified monooxygenase system to yield trans-5,6-dihydrodiol and trans-8,9dihydrodiol as major metabolites. Trans-3,4-,-10,11-,-1,2dihydrodiols are also formed in small amounts (Sims, 1970; Thakker et al., 1979). In general, B[a]A and its metabolites are considered to be weak carcinogens. However one metabolite, the trans-3,4-dihydrodiol, is known to possess mutagenic and tumorigenic capabilities. This congener has been observed to be oxidized by the cytochrome P-450 monooxygenase system to products that are 10 times more mutagenic in Salmonella typhimurium TA 100 than are benz[a]anthracene or any of its other dihydrodiol derivatives. Based on mutagenic, tumorigenic, and carcinogenic evidence, the trans-3,4-dihydrodiol-1,2epoxides (bay region diol epoxides) are indicated to be the ultimate carcinogenic metabolites of benz[a]anthracene (Wood et al., 1977). This metabolite is formed on a three-sided peripheral indentation called a "bay region." The diol epoxide arises by further enzyme-catalyzed oxidation of the double bond adjacent to the existing trans-dihydrodiol. These results are in accord with studies on benzo[a]pyrene that identified a bay region dihydrodiol epoxide as this compound's ultimate carcinogen (Sims and Grover, 1981).

Metabolic activation of PAH by monooxygenation, which is formally a two-electron oxidation, involves formation of vicinal diol-epoxides as the ultimate carcinogen. Another major pathway which has recently become the focus of considerable interest involves one-electron oxidation, resulting in the formation of radical cations, which, like the diol-epoxides, can react with cellular nucleophiles (Miller and Miller, 1981, Cavalieri and Rogan, 1984).

One-electron Oxidation of PAH

A PAH radical cation is generated by removal of a m electron from the aromatic system. The ease of formation of this species is a function of the compound's first ionization potential (IP), which is the energy required for complete removal of an electron from the highest occupied molecular orbital (HOMO).

Cytochrome P-450 acting in the presence of NADPH and dioxygen is not considered to act primarily as a oneelectron oxidant, yet cases have been observed (Augusto et al., 1982; Rauckman et al., 1982). The one-electron pathway appears to be more efficiently catalyzed in the presence of hydroperoxide cofactors. It has been demonstrated that the oxidation of benzolalpyrene carried out by liver microsomes yields different product distributions depending on whether the hydroxylation is supported by NADPH or cumene hydroperoxide (Capdevila, Estabrook, and Prough, 1980). While the major products obtained with NADPH were phenols, presumably derived from epoxidation and rearrangement, the metabolite profile for cumene hydroperoxide showed a preponderance of three quinone isomers. The quinones were likely formed from a one-electron oxidation of the hydrocarbon, since these products are known to be derived also via non-enzymatic one-electron oxidations. These results suggest that the reaction mechanism operating in the presence of the organic hydroperoxide may differ from that functioning in an NADPH-supported oxidation. For this reason caution must be used in equating NADPH-supported reactions of cytochrome P-450 and hydroperoxide-dependent reactions of hemeproteins.

Peroxidases, which are enzymes that catalyze the oxidation of inorganic or organic substrates at the expense of a hydroperoxide, primarily catalyze electron transfer reactions. They are much less efficient in carrying out

aliphatic hydroxylation or olefin epoxidation, reactions that proceed via direct insertion of oxygen across C-C double bonds. Initial electron transfer occurs between substrate and enzyme, but subsequent reactions of the resulting cation radical may occur in solution, and the oxidation products will depend on the solution chemistry and the nucleophilic species present (Ortiz de Montellano, 1986).

One specific peroxidase, called lignin peroxidase, is produced by the fungus *P. chrysosporium*, and acts to degrade lignin, a plant polymer of randomly linked phenylpropane units. In the presence of H_2O_2 , lignin peroxidase catalyzes one-electron oxidations of lignin-related compounds, and due to its low substrate specificity, it was expected to oxidize aromatics and heteroaromatics as well. It was found that purified lignin peroxidase did indeed carry out one-electron oxidation of PAH that had ionization potentials \leq a value of 7.55 eV (Hammel et al., 1986).

The lignin peroxidase-catalyzed oxidation of the PAH pyrene was carried out in aqueous medium. Under these conditions, the expected one-electron oxidation products are the 1,6- and 1,8-quinones, resulting from nucleophilic attack of water at carbons possessing the highest positive charge density (Cavalieri and Rogan, 1985). Through gas chromatography and mass spectrometry analysis the identity of these products was confirmed, and $H_2^{18}O$ -labeling

experiments proved that water was the source of the quinone oxygens (Hammel et al., 1986).

Previous studies supported the involvement of lignin peroxidase in the initial degradation steps of benzo[a]pyrene to its expected quinone derivatives (Haemmerli et al., 1986). Once formed, the benzo[a]pyrene cation radical reacts with water to yield a mixture of 1,6-, 3,6-, and 6,12-diones. These results and those discussed above are consistent with lignin peroxidase plus H₂O₂ acting as a fairly non-specific oxidation reagent for aromatic pollutants, proceeding via a cation radical mechanism.

Horseradish peroxidase-mediated binding of PAH to DNA is another reaction known to occur by one-electron oxidation. Once formed, PAH radical cations bind covalently to nucleophilic sites of biological macromolecules including DNA. Again, studies of PAH with known ionization potential have shown that compounds must possess an IP below a specific threshold in order to be oxidized and activated by this mechanism (Cavalieri et al., 1983).

Irrespective of the fate of the cation radical, evidence has accumulated indicating that only PAH with relatively low IP can be activated biologically by oneelectron oxidation. The carcinogenicity of PAH with relatively high IP would be due to the formation of bayregion diol epoxides (Conney, 1982). The existence of a few inactive PAH with low IP indicate that this characteristic is necessary but not always sufficient. Examples of this

type of PAH include perylene and anthanthrene. These weak or inactive PAH are known to have their positive charge delocalized over several carbons, indicating highly localized charge is also a requisite for activation by this mechanism. Positive charge localized on one or two carbons renders those positions more reactive toward nucleophiles.

Catalytic Cycle of Cytochrome P-450

The active site of cytochrome P-450 contains a single iron protoporphyrin IX prosthetic group, and it is here that dioxygen is bound, reduced and activated. An overview of its catalytic cycle is shown in Fig. 7, and includes the following steps: 1) Binding of substrate, resulting in a high spin ferric complex ; 2) One electron reduction of iron(III) to iron(II); 3) Binding of dioxygen; 4) A second one electron reduction; 5) Heterolysis of the O-O bond and concomitant production of the active oxidizing species and H_2O ; 6) Two electron oxidation of substrate producing oxidized product and returning the enzyme to its resting ferric state (Ortiz de Montellano, 1986).

Within this cycle, it has been found that the binding and reduction of molecular O_2 can be circumvented by the addition of exogenous monooxygen donors such as hydrogen peroxide, iodosobenzene and sodium periodate. The active oxidizing species derived via the peroxide shunt pathway is thought to be analogous to that formed by the reduction of dioxygen. For this reason, organic peroxides have been used



Fig. 7. An overview of the catalytic cycle of cytochrome P-450.



Fig. 8. Scheme showing ¹⁸O incorporation into norbornene oxide, proving the oxygen was exchangeable with added H₂¹⁸O.

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as monooxygen donors for the study of oxidative reactions catalyzed by cytochrome P-450.

Although the active oxidizing species of cytochrome P-450 has never been observed, the stoichiometry leading to dioxygen activation and the shunt pathway using monooxygen donors are identical to the chemistry characterized for peroxidase enzymes. Therefore, insight into the nature of the active oxidizing species has been sought through knowledge of an extensively characterized peroxidase, namely horseradish peroxidase (HRP). This protein reacts with H202 to generate an enzymic intermediate (HRP I or compound I) that is a monooxygen complex two electrons oxidized above the ferric resting state. The accepted view is that the two-electron oxidation of the enzyme to compound I involves heterolysis of the peroxy 0-0 bond, with one electron removed from the iron atom, the second electron abstracted from the porphyrin ring to form a n-cation radical. The resulting chemical species can thus be described as an oxoferryl porphyrin a cation radical. Compound I abstracts one electron from the substrate to form a compound II intermediate, which is subsequently reduced back to the resting enzyme by an additional one-electron oxidation of a second molecule of substrate (Van Wart and Zimmer, 1985).

Like horseradish peroxidase, the catalytic cycle of cytochrome P-450 is thought to involve a ferryl ion species as the active oxygen transfer agent. Support for the formation of this species is derived from observations that

peroxy acids and other single oxygen donors effect oxygen transfer in a manner similar to the fully reconstituted enzyme system.

Metabolism of PAH in mammalian, bacterial and fungal systems involves a wide variety of enzymes and cofactors and isozymes of cytochrome P-450 (Cerniglia, 1984). Comparing enzymatic product profiles to those obtained in biomimetic oxidations using synthetic catalysts has limitations. Nevertheless, the fundamental aspects of the mechanism of action of cytochrome P-450 can be elucidated through the use of porphyrin catalysts that exhibit similar reactivity.

Model Oxidations using Synthetic Catalysts

Synthetic tetraarylporphyrins have been used extensively to model the natural heme prosthetic group of cytochrome P-450. The bulky aryl substituents protect the meso carbons of the porphyrin ring from oxidation, and reduce the likelihood of heme agglomeration in solution. That these synthetic catalysts could carry out oxidations in the same manner as cytochrome P-450 was an important detail that had to be established. The first evidence came from Groves and Nemo (1979), where they described oxidative reactions of alkenes catalyzed by porphyrin complexes. It was demonstrated that iodosylbenzene reacted readily with iron porphyrin species in the presence of olefins to produce epoxides and alcohols in good yield.

In a later study by the same investigators, the preparation and characterization of the high-valent iron porphyrin complex was reported (Groves and Nemo, 1981), using the sterically encumbered porphyrin complex iron tetramesityl-porphyrin chloride [Fe(TMP)Cl]. Employing mchloroperoxybenzoic acid as oxygen donor, Fe(TMP)Cl was oxidized and converted to an apparent green intermediate, indicative of compound I analog formation. NMR, visible, Mössbauer and EPR data of the intermediate lend support to an iron(IV) porphyrin cation radical formulation. In addition, in the presence of H2¹⁸0, the unlabeled oxygen of the catalytically active complex was exchangeable with the heavier isotope, as indicated by 99% incorporation of 180 into the product epoxide (Fig. 8). These results are consistent with an oxoiron intermediate, and tend to disprove a free or metal coordinated peroxyacid as the active intermediate.

A more recent study explored preferential oxidation of the two isomers of stilbene using various synthetic porphyrin catalysts, indicating a marked *cis-trans* selectivity (Groves and Nemo, 1983). While *cis-stilbene* reacted to produce *cis-stilbene* oxide, *trans-stilbene* was found to be unreactive. This selectivity was postulated to result from nonbonding interactions between the phenyl rings of *trans-stilbene* and the iron porphyrin phenyl groups. The degree of *cis/trans* selectivity was dependent on the extent and nature of substituents on the aryl periphery of the



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Fig. 9. Proposed scheme for the epoxidation of olefins carried out by Fe (TMP)Cl and iodosylbenzene.



Fig. 10. Postulated approach for the iron-oxo group and the olefin substrate.

porphyrin, and is a good indication that oxygen transfer must have occured at or very close to the iron center as would be expected for an iron-oxo intermediate (Fig. 9). As established in previous work (Groves and Nemo, 1981), the iron IV porphyrin cation radical is the high valent species involved in the epoxidation of the olefins in question. Based on a mechanism derived from molecular orbital theory, stereoelectronic effects on the oxygen transfer from iron to the C-C double bond have been postulated to arise from a side-on approach of substrate to allow favorable overlap between the p orbitals of the Fe-bound oxygen and the olefin (Fig. 10).

Investigations such as these emphasize the importance of considering steric interactions between the synthetic catalyst and organic substrate when evaluating product distributions.

A study by Gold et al., 1988, investigated the oxidation of benz[a]anthracene in an mCPBA/porphinatoiron system where the peroxyacid was proven to be unreactive in the absence of catalyst. It was observed using 2,4,6trimethoxyphenyl-, and 2,6-dichlorophenyl-substituted catalysts that B[a]A was oxidized predominantly to the 7,12quinone. These results lend support to oxidation of the PAH *meso* carbons via charge transfer. Taking steric considerations into account, oxygen transfer via electrophilic substitution at these positions may not be

feasible, and a charge transfer oxidation pathway would have to be followed.

As indicated in the description of the enzymatic profiles, there is no evidence of fungal, bacterial, or mammalian enzymatic oxidation at the two meso carbons. The difference in the product profiles could be the result of a lower oxidation potential for the cytochrome P-450 oxoferryl porphyrin cation radical than for the model complex. If the oxidation potential is below that required to react with the substrate via charge transfer, electrophilic substitution would be the preferred pathway for the enzyme.

Many biomimetic studies on model compounds are carried out in CH2Cl2. There is some uncertainty about the validity of these studies due to the present knowledge that compound I formation is not entirely clean when pure CH2Cl2 is used as a reaction medium. A polar co-solvent, such as deuterated methanol (CD3OD), is also required, and was used in the oxidations outlined in this report. This species adds polarity to an otherwise non-polar solvent system, facilitating ionization of the iron atom and exchange of the axial ligand, eventually resulting in compound I formation. In the case of our catalyst, (see Fig. 1), the axial ligand is the triflate anion (OSO2CF3), which is weakly coordinated to the iron, and electron-withdrawing chlorines are present on the phenyl rings. Both of these characteristics lead to facile compound I formation. The reaction scheme below (Fig. 11), depicts compound I analogue

formation via coordination of the peroxy acid, taking the place of chlorine (or triflate) as the axial ligand, and emphasizing the importance of ligand exchange in this process.

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Fig. 11. Exchange of the axial ligand leading to compound I analog formation.

CHAPTER III EXPERIMENTAL METHODS

Instrumentation and Lab Materials

¹H nuclear magnetic resonance (NMR) spectra were obtained at the UNC Department of Chemistry on a Varian XL-400 at 400 MHz. Chemical shift values are reported relative to tetramethylsilane (TMS). UV-visible spectra were recorded on a Spectronic 1201 spectrophotometer (Milton Roy Company). Mass spectral data were taken using a direct insertion probe on a VG 70S 250SEQ mass spectrometer in the EI mode at 70eV at the UNC Department of Environmental Sciences and Engineering.

Preparative thin layer chromatography (TLC) was performed using Silica Gel PK5F (20cm x 20cm) 500 µm plates (Whatman), while analytical TLC was conducted using silica gel on aluminum (Aldrich). Column chromatography was carried out using Merck Silica gel, grade 60, 230-400 mesh, 60%.

Benz[a]anthracene was commercially available (Aldrich) and was purified by column chromatography on silica using benzene/hexane as eluant. *m*-Chloroperoxybenzoic acid was obtained from Fisher Scientific Co. and used as received. ¹⁸0-mCPBA was prepared by photolysis of *m*-chlorobenzaldehyde in the presence of ¹⁸0 in a CH₂Cl₂ medium at -60°C. FeTPP(2,6-Cl)OSO₂CF₃ was synthesized as described in the methods section. Reagent grade solvents were employed, and were dried and distilled prior to use where indicated. Any routine chemicals were purchased from one of the following sources: American Scientific Products; Aldrich Chemical Company, Inc. ; Sigma Chemical Company; and Fisher Scientific.

Porphyrin Synthesis

1) Synthesis of TPP(2, 6-C1)H2

This reaction was carried out under argon atmosphere by evacuating a manifold system and filling with argon. 1.6 L of CH₂Cl₂ (0.2% ethanol, 3.2 mL added as preservative) was placed in a 3-necked flask with reflux condenser and two septa attached. 2,6-Dichlorobenzaldehyde (2.8 g, 16 mM) was added before 1.1 mL distilled pyrrole (16 mM) was placed into the reaction mixture through a septum via syringe. Finally, 1 mL BF₃-etherate was added via syringe and stirred 2 hours to form the porphyrinogen (Hoffman et al., 1990).

The UV spectrum was checked after 2 hours (max=511 nm, shoulder at 490 nm corresponding to porphyrinogen). After addition of *p*-chloranil (2.85 g), the reaction was refluxed for 2.5 hours to obtain the porphyrin free base TPP(2,6-C1)H₂ (514 nm, 416 nm). The free base was allowed to precipitate out of reaction mixture and was filtered through a medium frit to obtain a solid, which was subsequently purified using an aluminum oxide column with CH₂Cl₂ as eluant. Fractions that were pure by UV spectra and analytical TLC were pooled to give a final yield of 430 mg (11%).

2) Metallation of TPP(2,6-C1)H2

The solution of 210 mg free base in 85 mL DMF under argon was heated to reflux and excess FeCl₂.4H₂O was added; this proceeded approximately 17 hours overnight. The next day an additional 100 mg FeCl₂ was added and the reaction heated 30 minutes, then refluxed under air for 2 hours.

DMF was distilled off under vacuum using a liquid nitrogen collecting trap, and the remaining solid redissolved in a minimal amount of CHCl₃ and chromatographed on a silica column using CHCl₃ as eluant. A band was collected containing a mixture of TPP(2,6-Cl)FeCl and TPP(2,6-Cl)FeOH. To convert any OH complex to chloro complex, H₂SO₄ was dropped onto NaCl, generating HCl gas which was bubbled through a solution of the iron porphyrin in 200 mL CH₂Cl₂. The solvent was evaporated on a rotary evaporator, and the solid was dried and weighed. Yield was 180 mg FeTPP(2,6-Cl)Cl (86%).

3) Synthesis of FeTPP(2,6-C1)OSO2CF3

90 mg FeTPP(2,6-Cl)Cl dissolved in 30 mL dry tetrahydrofuran (THF) was added to 100 mg silver triflate in

6 mL THF in a Schlenk tube in a glove box under argon. FeTPP(2,6-Cl)Cl has a characteristic UV spectrum with max=418 nm and 508 nm. After 3 hours, the UV spectrum was taken (max=395 nm, 511 nm) which corresponds to FeTPP(2,6-Cl)OSO₂CF₃. The THF was removed by bulb-to-bulb distillation using a liquid nitrogen trap. The residue was returned to the glove box, dissolved in a minimal amount of dry CH₂Cl₂, and filtered to remove insoluble silver salts. The solvent was removed, yielding 102 mg of pure triflate complex (98.9%). This species was subsequently used in all oxidations where a porphyrin catalyst was employed.

Oxidations Using Porphyrin Catalysts

Oxidation with the green Compound I analog

This oxidation was carried out at -80°C in an ethanol/dry ice bath under argon atmosphere. 16 mg m-chloroperoxybenzoic acid (.09 mmoles) in 0.5 mL CD₃OD was added to 16 mg FeTPP(2,6-C1)Tf (.02 mmoles) in 2 mL CH₂Cl₂ to generate the green compound I analog (oxoferryl porphyrin cation radical). B[a]A (50 mg, .22 mmoles) in 2 mL CH₂Cl₂ was added, and a color change from green to brown was observed. After 1 h, 5 mL saturated Na₂CO₃ solution was added to neutralize the mCPBA, CHCl₃ was added, and the organic layer was extracted and dried over anhydrous sodium sulfate. The solvent was evaporated on a rotary evaporator at 45°C and the resulting reaction mixture (dissolved in a minimal amount of CHCl₃) was applied to preparative TLC plates (500µm) to separate the oxidation products.

Catalytic or Multi-turnover Oxidations

These oxidations used an approximate 100-fold excess of mCPBA over the porphyrin catalyst to generate multi-turnover conditions. Again 40-50 mg B[a]A and 7 mg FeTPP(2,6-Cl)Tf (.006 mmoles) were stirred together at -80°C under argon before 80 mg mCPBA (.46 mmoles) was added to serve as oxygen donor. The reaction was allowed to proceed for 1 hour and the subsequent workup was identical to the other oxidative mode in which the porphyrin was used as catalyst.

Acetylation of Oxidation Reaction Mixtures

Due to the observed instability of some phenols and dihydrodiols which prevented accurate characterization of the reaction products, the reaction mixtures were acetylated following oxidation. Through the addition of pyridine and acetic anhydride any phenols or dihydrodiols formed are acetylated and stabilized.

The oxidation workup was amended as follows: After reaction completion, before the addition of bicarbonate, the solvent was removed by blowing reaction mixture with argon. The residue was redissolved in a minimal amount of pyridine and an equal volume of acetic anhydride (1:1 volume ratio) was added, and the mixture was allowed to stir overnight. The reaction was quenched with bicarbonate, and the same subsequent steps were followed to obtain the reaction product mixture.

One-electron Oxidations

Preparation of Phenoxathiin hexachloroantimonate

This reaction was performed at room temperature in the glove box under argon atmosphere, following the procedure of Gans et al, 1981. 600 mg of antimony pentachloride in 10 mL dried CH₂Cl₂ was added to a solution of 500 mg phenoxathiin in 15 mL CH₂Cl₂. The reaction mixture was allowed to stir for 15 minutes, during which time the cation radical hexachloroantimonate precipitates as a violet microcrystalline powder. The solution was filtered and dried and the solid was weighed. Average yield was 830 mg oxidant (75%).

Oxidation using Phenoxathiin hexachloroantimonate

This reaction was performed at room temp. under argon atmosphere. An equimolar amount of phenoxathiin hexachloroantimonate (62 mg, .12 mmoles) in 5 mL dry CH₂Cl₂ was added to 27 mg B[a]A (.12 mmoles) in 5 mL CH₂Cl₂. The reaction was quenched after 30 min. with 10 mL methanol, and the solvent was removed at 55°C on a rotary evaporator. The resulting solid mixture was dried under oil pump vacuum, and separation of the oxidation products was carried out using preparative TLC on SiO₂ developed in CHCl₃.

CHAPTER IV RESULTS

The NMR and UV-visible spectra of the B[a]A derivatives were compared to those of the parent compound. The ¹H NMR spectrum of benz[a]anthracene in CDCl₃ was taken after purification using column chromatography (Fig. 12), and its UV-visible spectrum is shown in Fig 13.

Oxidation of B[a]A using Fe(IV) cation radical as direct oxidant

The first oxidations were performed in this mode; before acetylation was used as a method to stabilize the oxidation products. Separation of products was carried out using column chromatography, and only two fractions were definitively characterized. Fraction 2 was identified as 7,12-OCD₃-B[a]A on the basis of the following data: FAB Mass Spectrum (see Fig. 14a) - m/z 295 (MH)⁺; 262 (MH-OCD₃)⁺; 228 (MH-OCD₃-OCD₃)⁺. ¹H NMR (400 MHz, CDCl₃) (see Fig. 14b) - 6 8.39 (1H, d, J=8.80 Hz, H1); 7.34-7.88 (9H, m, H2-H6, H8-H11). The absence of singlet resonances in the ¹H NMR spectrum is key to the assignment of substitution at







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the C7 and C12 positions. Fraction 3 was confirmed to be 7-OCD₃-12-OH-B[a]A on the basis of the following: FAB Mass Spectrum (see Fig. 15a) - m/z 278 (NH)⁺; 244 (MH-OCD₃)⁺; 226 (MH-OCD₃-OH)⁺. ¹H NMR (400 MHz, CDCl₃) (see fig. 15b) - δ 8.73 (1H, d, J=9.20 Hz, H1); 8.4-7.58 (9H, m, H2-H6, H8-H11). As in the case of fraction 2, the C7,C12 substitution is based on the absence of singlet resonances in the ¹H NMR. The UV-visible spectra of Fractions 2 and 3 are shown in Fig. 16a and b, respectively.

Oxidations followed by acetylation in this mode were performed twice as described in Chapter III. After work-up the products were separated on two preparative 500 µm TLC plates developed in CHCl3. While mostly unreacted B[a]A was recovered as the first band (63% and 86% for the first and second experiments), the predominant amount of product was collected as band 3 (20% and 7%, respectively, based on mCPBA added). The remaining bands accounted for trace percentages. The lover yield of the second oxidation resulted from the use of a two-fold excess of mCPBA over the porphyrin compared to an initial 8-fold excess. The assignment of band 3 as a mixture of 8-acetoxy-BlalA and 11acetoxy-B[a]A was based on the following spectral data. 1H NMR (400 MHz, CDC13) (see Fig. 17) - & 9.19 (2H, s, H12 & H12'); 8.81 (1H, d, J=8.34 Hz, H1); 8.77 (1H, d, J=8.81 Hz, H1'); 8.39 (1H, s, H7); 8.35 (1H, s, H7'); 8.02 (1H, d, J=8.41 Hz, H8 or H11); 7.93 (1H, d, J=8.48 Hz, H11 or H8); 7.35-7.85 (14H, m, H2(H2')-H6(H6'), H9(H9'), H10(H10'). EI



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Mass Spectrum (see Fig. 18) - m/z 286 (M)*; base peak 244 (M-[O=C=CH₂]); 215 (M-acetate). Its UV-visible spectrum is shown in Fig. 19. The identification of the band as a mixture of 8- and 11-acetoxy isomers rests on the presence of two singlets in the region of H7 and two acetate methyl singlets.

Oxidation of BlalA using 180-mCPBA

To prove that the acetoxy derivatives stem from monooxygen transfer from mCPBA to the iron porphyrin species, ¹⁸O-mCPBA was employed in the following oxidations, and its incorporation was measured by EI mass spectrometry.

B[a]A (30 mg, .13 mmoles) in 1.5 mL CH₂Cl₂ was added to the oxoferryl porphyrin cation radical generated by mixture of ¹⁸O-mCPBA (6.5 mg, .04 mmoles) in 0.5 mL CD₃OD with FeTPP(2,6-Cl)Tf (16 mg, .02 mmoles) in 3 mL CH₂Cl₂. The green color that was apparent in the previous oxidations was not as markedly apparent, so 3.5 mg more of the peroxy acid was added. This reaction was not expected to be as efficient due to the lower known activity of labelled mCPBA, as indicated by titration experiments with iodine, and smaller yields were predicted. The reaction was allowed to proceed 1 h at -80°C under argon, then acetylated overnight. The reaction was worked up following standard protocol, and the two predominant oxidation products were weighed (Bands 2 and 3; 4% and 8% yield, respectively). Band 2 was identified as 7-OCD₃-B[a]A on the basis of its EI mass spectrum (data not shown). The presumed acetoxy mixture (Band 3) was submitted for mass spectral analysis to determine ¹⁸0 vs. ¹⁶0 incorporation.

EI Mass Spectrum of Band 3 (see Fig. 20a) - m/z 286, 288 (M)⁺ (B(a)A-160, 180-acetate); 244, 246 (M-160, 180=C=CH₂). (M)⁺ peaks present in a 1:1 ratio indicating 50 % ¹⁸0-acetate and 50 % ¹⁶0-acetate. It is possible that during acetylation, nucleophilic exchange of 180-acetate with ¹⁶0-acetate from the acetic anhydride occured. To determine whether or not this was the case, the oxidation was repeated, but the acetylation was carried out for a much shorter period.

BIalA (25 mg, .11 mmoles) was oxidized using the same amounts of mCPBA and catalyst as before. Acetylation was performed using acetyl chloride and pyridine, and the pyridine salt precipitated out so rapidly that the reaction mixture could not be stirred. Comparable yields were obtained for this experiment. Again, band 2 was identified as 7-OCD₃-BIalA on the basis of its mass spectra (data not shown). The EI mass Spectrum of Band 3 (see Fig. 20b) yielded ¹⁸O and ¹⁶O acetoxy peaks in a 2:1 ratio, indicating a higher proportion of acetoxy derivatives that contain ¹⁸O from the labelled mCPBA, supporting the hypothesis that exchange was occuring.



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Catalytic oxidation of Benz[a]anthracene

These experiments were done according to the procedure described, and the oxidation products were resolved using preparative TLC. Yields were determined based on moles of hydrocarbon used as substrate, which was the limiting reactant. As shown by the higher yields and lower amount of unreacted B[a]A recovered, these oxidations were more efficient than those using the oxoferryl porphyrin cation radical in a single turnover. The major fraction collected was Band 2, which migrated very close to the parent compound. Yields of 25% and 63% for this derivative in the first and repeat experiment were obtained. The higher efficiency of the repeat experiment is unexplained, since identical amounts of reactants were used. Band 2 was confirmed to be 7-OCD3-B(a)A on the basis of the following spectral data: ¹H NMR (400 MHz, CDCl₃) (see Fig. 21) - 6 8.97 (1H, s, H12); 8.81 (1H, d, J=8.41 Hz, H1); 8.28 (1H, dd, J=6.5, 2.91 Hz, H11); 8.14 (1H, d, J=9.7 Hz, H6); 8.11 (1H, dd, J=6.21, 2.91 Hz, H8); 7.84 (1H, d, J=8.65 Hz, H4); 7.66 (1H, d, J=9.31 Hz, H5); 7.50-7.65 (4H, m, H2, H3, H9, H10). EI Mass Spectrum (see Fig. 22) - m/z 261 (M)*; 243 (M-CD₃); 228 (M-CD₃-O); 215 (M-CD₃-O-C); Accurate Mass of 261.1237 observed within 1.4 ppm of mass calculated for the elemental composition C19H11D30 (see Fig. 23). Its UV spectrum is shown in Fig. 24a. Absence of a singlet





Pid :

Fig. 22. EI Mass Spectrum of 7-OCD3-Benz[a]anthracene.

Elemental Composition

Heteroatom Ma Limits:	x: 20	Ion: 1	Both Even and	odd					
•				-0.5	15	5	0	0	0
261.123656	10.0			20.0	25	20	5	3	3
Mass	mDa.	PPM	Calo. Mass	DBE	c	п	28	ы	0
261.123656	0.2	-0.6	261.123501	14.0	17	11	2	3	
	-> -0.4	-1.4	261.123295	14.5	19	11	3		14-
	1.2	4.5	261.124844	13.5	19	13	2		1
	1.4	5.3	261.125049	13.0	17	13	1	3	
	-1.7	-6.5	261.121953	15.0	17	9	3	3	
	-1.9	-7.3	261.121747	15.5	19	9	4		1
	2.7	10.5	261.126392	12.5	19	15	1		1
	2.9	11.3	261.126598	12.0	17	15		3	
	3.3	-12.5	261.120404	16.0	17	7	4	3	
	-3.5	-13.2	261.120199	16.5	19	7	. 5		1
	4.3	16.4	261.127940	11.5	19	17			1
	-4.8	-18.4	261,118856	17.0	17	5	5	3	
	5.1	19.5	261,128752	12.0	15	9	5	1	3
	6.6	25.4	261.130301	11.0	15	11	. 4	ī	3
	7.8	29.8	261,131432	16.5	18	. 7	5	2	
	8.2	31.4	261,131849	10.0	15	13	3	1	3
	-8.3	-31.8	261.115364	12.0	18	15		ī	i
	9.3	35.7	261,132981	15.5	18	9	4	2	
	9.7	37.3	261.133397	9.0	15	15	2	ī	3
	-0.0	-37.7	261 112016	11 0	10	12		1	



Fig. 23. Accurate Mass analysis for 7-OCD3-Benz[a]anthracene.



resonance corresponding to H7 in the ¹H NMR requires that the substitution occur at C7.

Phenoxathiin hexachloroantimonate Oxidation

This reaction was carried out as described in Chapter III; after quenching the reaction with methanol, the oxidation products were separated on two preparative 500 µm TLC plates developed in CHCl3. Predominant band scraped from plate was Band I (23.5 mg according to NMR integration relative to phenoxathiin, 87% yield). The assignment of Band I as 7-Cl-benz[a]anthracene was confirmed by the following: ¹H NMR (400 MHz, CDCl₃) (see Fig. 25) - & 9.04 (1H, s, H12); 8.74 (1H, d, J=8.01 Hz, H1); 8.49 (1H, d, J=8.66 Hz, H11); 8.31 (1H, d, J= 9.38 Hz, H6); 8.06 (1H, d, J=8.09 Hz, H8); 7.83 (1H, d, J=7.77 Hz, H4); 7.71 (1H, d, J=9.45 Hz, H5); 7.50-7.70 (4H, m, H2, H3, H9, H10). Before the sample was submitted for EI analysis, the substituted hydrocarbon was crystallized out of methanol to separate it from the phenoxathiin, which remained in solution. EI Mass Spectrum (see Fig. 26) - m/z 262, 264 (M)+; 226 (M-HC1); 200 (M-HC1-C2H2 group). The ratio of m/z 262: m/z 264 is 3:1, correct for the expected 35C1:37C1 isotopic distribution. UV-visible spectrum is shown in Fig. 24b.





Fig. 26. EI Mass Spectrum of 7-C1-Benz[a]anthracene.

CHAPTER V DISCUSSION AND CONCLUSION

Identification of predominant fractions obtained from the oxidations was used as an indication of the major pathways involved. Electron abstraction and additionrearrangement are both expected to yield specific oxidation products based on the charge distribution properties of benz[a]anthracene. Highest positive charge of PAH cation radicals is localized at the PAH meso positions (C7 and C12), and substitution at these carbons is support for the one-electron oxidation pathway. Electrophilic attack is expected at relatively localized arene bonds (such as C5-C6). A table summarizing the products obtained from the three different types of oxidations is shown in Table 1.

Initially, the oxidation products of benz[a]anthracene under single turnover conditions were not stabilized by acetylation and were separated by silica column chromatography. As the column was eluted, B[a]A derivatives were screened using UV-visible spectroscopy. However, as we learned from later results, the UV spectra of monosubstituted oxidation products, with the aromatic system still intact, are not necessarily significantly different



from the parent hydrocarbon. Even though no phenol derivatives were isolated or identified, they may have been present and pooled with the unreacted B[a]A on the basis of their UV spectra. Two fractions were characterized. 7,12-OCD₃-B[a]A resulted from one-electron oxidation of the PAH and nucleophilic attack of OCD₃, probably first at C7, followed by a second oxidation with attack at C12. 7-OCD₃-12-OH-B[a]A was probably formed from initial oxidation to 7-OCD₃ product followed by a second oxidation resulting from exposure to air during workup. Acetylation, which was carried out in subsequent oxidations, not only stabilizes any phenol or dihydrodiol derivatives, but ensures that the derivatives characterized were formed under the specified conditions (an argon atmosphere with mCPBA the only source of oxygen).

Oxidation by the known one-electron oxidant phenoxathiin hexachloroantimonate was carried out to confirm that substitution would occur at C7 (as predicted by HOMO theory). As predicted, the NMR and mass spectral data confirm that in a CH₂Cl₂ medium, the cation radical abstracts an electron from benz[a]anthracene, and the chloride ion (Cl⁻), acting as a nucleophile, attacks the carbon possessing the greatest positive charge. Formation of 7-Cl-B[a]A resulted in a stable species and further oxidation did not occur.

Oxidation in the single turnover mode produced predominantly 8- and 11-acetoxy-B[a]A when acetylation was

carried out. Whether these derivatives were the result of one-electron oxidation or electrophilic attack is hard to determine. The latter pathway involves formation of an epoxide which was not demonstrated (but could be through observation of an NIH shift). However, one-electron oxidation followed by oxygen rebound could also proceed via an epoxide intermediate, a possibility that is considered below in greater detail. There were no products identified resulting from substitutions at C5 or C6, representing oxidation of the most localized bond, yet it could be the case that steric interactions arising from the spatial relationship between the substrate and porphyrin would not favor oxidation at these sites.

We favor the postulate that the same oxidative pathway, namely one-electron oxidation, is operating in the single and multi-turnover modes, and that reaction condition variables account for the difference in product profiles. Since the charge distribution of the B(a)A radical cation and any steric constraints should apply equally in both single turnover and catalytic cases, the difference in product profiles between the two porphyrin modes can be thought of in terms of the relative concentrations of substrate and catalyst.

In the case of the single turnover oxidations, the approximately equal concentration of porphyrin relative to substrate without excess mCPBA favors the porphyrin-PAH complex to remain associated for a sufficient time to allow

oxygen rebound to occur. The regiochemistry of cage recombination of the oxoferryl porphyrin cation radical with the PAH cation radical is influenced by the requirement of side-on approach for oxygen transfer. In the case of benz[a]anthracene, the carbons at the terminal ring of the molecule (C8-C11), may be the only region accessible to oxidation (see Fig. 27). The initial product of oxygen rebound can be formally considered to be an 8,9- or 10,11oxide which will rearrange to an 8- or 11-phenol, respectively (see. Fig. 28). Acetylation yields the corresponding acetoxy derivative.

The catalytic oxidations used a lower concentration of porphyrin relative to substrate with an excess of mCPBA, leading to more competitive complexation and consequently to a transient porphyrin/PAH complex. The PAH cation radical may be formed as in the single turnover mode, but may escape from the cage before oxygen rebound occurs, leading to products of attack by nucleophiles in the reaction medium. In the CH₂Cl₂/CD₃OD solution, this results in attack of methanol-d₄ at C7, which is the most electrophilic site on the periphery of the benz[a]anthracene cation radical, yielding 7-methoxy-d₃-benz[a]anthracene. In agreement with this theory, the major product formed was 7-OCD₃-B[a]A.

The ¹⁸O-labeling experiments proved that the oxygen incorporated into the substrate in the single turnover oxidations was derived from ¹⁸O-mCPBA. The peroxyacid forms a complex with the iron porphyrin, heterolysis of the ¹⁸O-



Fig. 27. Proposed orientation of B[a]A when approaching iron porphyrin.

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180 bond occurs, and an [180]-oxo-iron intermediate is formed which is the active oxidizing species (see Fig. 29).

Hydrophobic and hydrophilic characteristics of residues at the active site of cytochrome P-450 govern how a specific substrate would interact with the enzyme. The substituted porphyrin that we employ as catalyst is a scant model relative to a complex protein. Although definitive conclusions concerning monooxygenase activity of the iron porphyrin cannot be drawn from these experiments and applicability of these specific results as a model for the reactivity of cytochrome P-450 in a biological system may be uncertain, this work is the first systematic examination of PAH oxidation by the compound I analogue thought to be operative in cytochrome P-450-mediated monooxygenations.



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SUMMARY

We have carried out oxidations of benz[a]anthracene using a well-characterized high-valent iron porphyrin complex, and have repeated the oxidations under multiturnover conditions in which the high-valent species is not an observable intermediate. We have identified one-electron oxidation as the major pathway operating under multiturnover, truly catalytic conditions. Differences in product profiles under the single turnover and catalytic modes are interpreted in terms of kinetic effects resulting from the changes in reaction conditions. Further experiments should be pursued using other high-valent iron porphyrin species with varying degrees of substitution, different substituents on the phenyl rings, and different oxidation potentials. In addition, the oxidations should be performed using a variety of polycyclic aromatic hydrocarbons to gain further knowledge about structure/reactivity relationships between the synthetic porphyrin catalysts and their substrates.

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