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Porphyrin-Catalyzed Oxidation of Trichlorophenol

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ABSTRACT

Porphyrin–metal complexes are potentially useful to catalyze redox reactions, which convert toxic and biologically recalcitrant compounds to compounds that are less toxic and more amenable to biotreatment. Porphyrins, in the absence of proteins as in ligninases, peroxidases, and oxidases, are potentially more robust than enzymes and microbial cultures in the treatment of inhibitory substances.

2,4,6-Trichlorophenol was used as a model compound for chlorinated phenols and as a substrate for various porphyrin-metal complexes acting as oxidation catalysts. *t*-Butyl hydroperoxide was the oxidizing agent. TCP was shown to be at least partially dechlorinated and the aromatic ring broken in reaction products. All porphyrins exhibited saturation kinetics with regard to the initial TCP concentration in reaction mixtures. Electron-withdrawing substituents on the porphyrins were observed to increase stability of the catalysts to inactivating ring-centered oxidation.

Index Entries: Trichlorophenol; porphyrin; biomimetic; heme; dechlorination.

INTRODUCTION

Extensive use of chlorinated phenols, such as pentachlorophenol, as fungicides and wood preservatives has resulted in significant contamination of ground water, surface water, and soils with these compounds. Chlorinated phenols are also found in the effluents of Kraft paper mills formed as a result of lignin degradation *(1)*. The toxicity of chlorophenols in aquatic systems and to humans is well established *(2–4)*.

Many microorganisms have been shown to mineralize pentachlorophenol. However, the same organisms do not necessarily degrade other struc-

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turally similar chlorinated phenols. Mixtures of chlorinated phenols can escape treatment because of crossinhibition *(5)*. Anaerobic/aerobic mixed cultures in series have been shown to mineralize several chlorinated phenols *(6)*. The maintenance of two dissimilar cultures in series clearly adds a level of complexity to the process. The white-rot fungus *Phanerochaete chrysosporium* has been shown to degrade a variety of chlorinated phenols via a system of exogenous, nonspecific peroxidases or ligninases *(7,8)*. However, these ligninases are produced only when the organism is starved for a key nutrient like nitrogen *(9)*. Therefore, the utilization of this organism for treatment of chlorinated phenols and other substituted aromatics requires a feed-starve approach to maintain viability and ligninase production.

Several investigations have been demonstrated that porphyrins alone, in the absence of protein as in ligninases, can catalyze oxidation and reduction reactions of lignin model compounds. Porphryin–metal complexes have been shown to catalyze the reductive dehalogenation of halogenated hydrocarbons and the reduction of nitroaromatics to corresponding amines *(10–13)*. Shimada et al. *(14)* have also reported the oxidative cleavage of C—C bonds in lignin model compounds catalyzed by Fe^{+3} protoporphyrin IX in the presence of an active oxygen donor, such as *t*-butylhydroperoxide (TBHP). Hickman et al. *(15)* have reported that oxidation of metalloporphyrins may be metal-centered or ring-centered. Ring-centered oxidation results in inactivation of the porphyrin as a catalyst. Metal-centered oxidation allows the oxidized metal ion to abstract an electron from a susceptible functional group and be reduced to the original oxidation state. Nanthakumar and Goff *(16)* have shown that porphyrins with electronegative substituents in the *o*-aryl positions are more stable toward ring-centered oxidation and, therefore, conceivably more useful as redox catalyst.

Porphyrin–metal complexes are potentially useful to catalyze redox reactions, which convert toxic and biologically recalcitrant compounds to compounds that are less toxic and more amenable to conventional biological treatment. Porphyrins, in the absence of protein as in ligninases and other peroxidases and oxidases, are potentially more robust than enzymes and microbial cultures in the treatment of inhibitory substrates. In this work, 2, 4, 6-trichlorophenol (TCP) was used as a model compound for chlorinated phenols and as a substrate for various porphyrin–metal complexes acting as oxidation catalysts. The objective was the catalytic oxidation of TCP to less-chlorinated and less-toxic products.

MATERIALS AND METHODS

Chemicals

All porphyrins used in this work, including Fe^{3+}-protoporphyrin IX (Fe-pro-Ph), Fe^{3+}-meso-tetra(*o*-diclorophenyl) porphine sulfonate (Fe-diCl-Ph), and Fe^{3+}-meso-tetra(*o*-difluorophenyl) porphine sulfonate (Fe-diF-

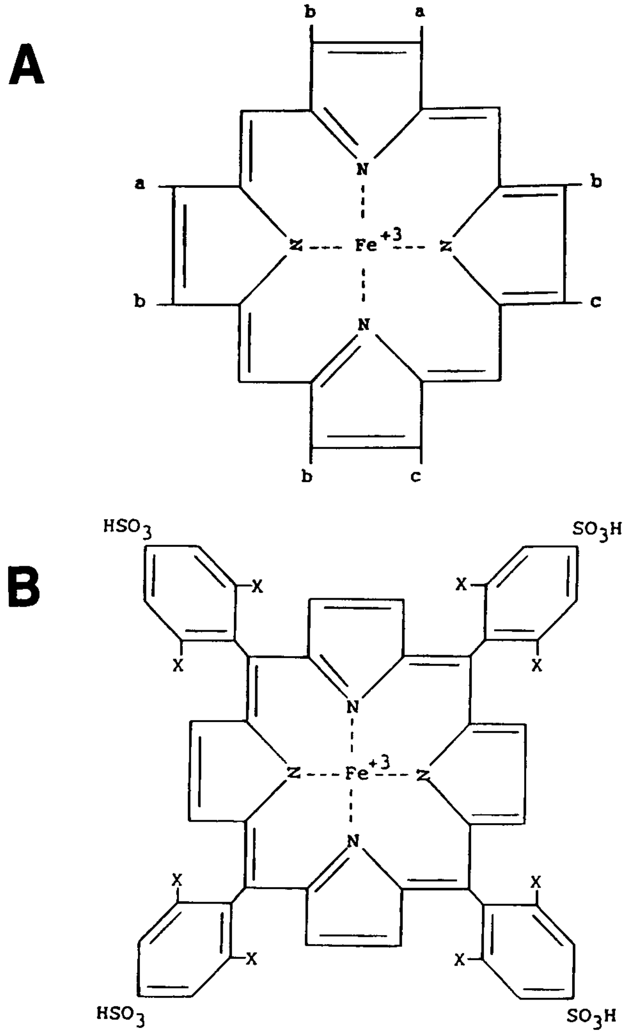

Fig. 1. Structures of **(A)** Fe^{3+}-protoporphyrin IX (a = —CH=CH; b = —CH_3; c = —CH_2CH_2COOH) and **(B)** Fe^{3+}-meso-tetra(*o*-dichlorophenyl)porphine sulfonate (x = Cl) and Fe^{3+}-meso-tetra(*o*-difluorophenyl)porphine sulfonate (x = F).

Ph), were obtained from Porphyrin Products (Logan, UT). TCP was obtained from Aldrich Chemical Co. (Milwaukee, WI). A 100 mg/L standard of TCP in methanol was obtained from Chem Service (West Chester, PA) and TBHP was obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals used were reagent-grade.

Porphyrin-Catalyzed Oxidation of TCP

Three porphyrins were used in this study, Fe-pro-Ph, Fe-diCl-Ph, and Fe-diF-Ph (*see* Fig. 1). Fe-pro-Ph was chosen as an example of a porphyrin susceptible to ring-centered oxidation. Fe-diCl-Ph and Fe-diF-Ph both have electronegative substituents in the *o*-aryl positions and should be more stable toward ring-centered oxidation. The sulfonate groups enhance water solubility.

Stock solutions of porphyrins were prepared in 0.1*M* phosphate buffer at pH 7.0 and stored in flasks covered with aluminum foil to prevent exposure to light. Porphyrin concentrations were verified using a calibration curve based on UV-VIS absorbance at a characteristic wavelength of each porphyrin. A stock TCP solution (5.0 m*M*) was also prepared in the 0.1*M* phosphate buffer at pH 7.0.

Reactions were typically conducted in 100-mL sealed septum bottles containing 3.0 m*M* TCP, 0.0010–0.0020 m*M* porphyrin, and 45 m*M* TBHP in a reaction volume of 35.0 mL. The temperature was 55°C and the pH was 7.0, which was shown to be optimal. The reaction was initiated by adding the oxidizing agent. Samples (1.0 mL) were removed periodically with a syringe and placed in 0°C/water bath to quench the reaction. Samples were analyzed for TCP, chloride, and other reaction products as detailed below. Control experiments were identical to the reaction experiments, except for the absence of porphyrin in the reaction mixture.

In order to determine the effect of pH on the reaction, a number of batch experiments were conducted at 55°C using Fe-diCl-Ph at various pH values. These experiments were conducted as described above, except that the pH was varied from 4.5–10.1. A 0.1*M* phosphate buffer was used at each pH. Sodium hydroxide was used to adjust the pH to the desired value. In each case, the pH remained constant during the course of the reaction. Other initial reaction conditions were as follows. Fe-diCl-Ph (0.0020 m*M*), TCP (1.5 m*M*) and TBHP (45.0 m*M*).

A comparison of the kinetics of TCP oxidation catalyzed by Fe-pro-Ph, Fe-diCl-Ph, and Fe-diF-Ph was conducted as follows. The effect of initial TCP concentration on the reaction catalyzed by each of the three porphyrins was conducted at a temperature of 55°C, pH 7.0, porphyrin concentration of 0.0010 m*M*, and a TBHP concentration of 45.0 m*M*. The initial TCP concentration was varied from 0.20–3.00 m*M*. Comparison of the amount of TCP oxidized/mol of porphyrin for different initial TCP concentrations was also used to demonstrate the catalytic nature of the reaction.

The effect of porphyrin concentration on the reaction rate was studied by conducting a series of TCP oxidation reactions using TBHP as oxidizing agent and Fe-diCl-Ph as the porphyrin catalyst. The temperature was 55°C, the TBHP concentration was 45.0 m*M*, and the initial TCP concentration was 1.6 m*M* in all the experiments, whereas the concentration of Fe-diCl-Ph was varied from 0.0–0.0014 m*M*.

A series of batch experiments was also conducted to study the effect of temperature on dechlorination of TCP. Reaction mixtures contained 2.1 m*M* TCP, 45.0 m*M* TBHP, and 0.0080–0.033 m*M* porphyrin. The reactions were conducted at 55, 70, and 100°C and pH 7.0, and were otherwise conducted as described above.

Analytical

A Hewlett Packard model 1090L high-pressure liquid chromatograph (HPLC), with UV-VIS diode array detector was used to measure TCP concentration in samples of the reaction mixture. The column used was (100 mm long and 46 mm id) Hypersil ODS (5 µm) and was supplied by Hewlett Packard (Palo Alto, CA). The solvent was methanol/water (45:55) at a flow rate of 1.5 mL/mm. The operating temperature was 40°C and the injection volume was 0.3 µL. Under these conditions, the retention times of TCP and TBHP were 4.0 and 1.0 min, respectively. A TCP standard solution (100 mg/L) from Chem Service (West Chester, PA) was used to quantify TCP concentration.

Chloride ion concentration in reaction mixtures was measured using an Orion Model 94-17B chloride electrode (Cole-Parmer Instruments, Niles, IL) using the method of standard addition. A 1000 mg/L standard chloride solution was also obtained from Cole-Parmer.

All reaction mixtures contained a fine precipitate that was filtered out and analyzed separately. A known volume of the reaction mixture was filtered through a membrane filter (pore size 0.45 µm) produced by Belman Instrument Co. (Ann Arbor, MI). The precipitate was dried and dissolved in 4.0 mL of HPLC-grade methanol.

Product identification was initially attempted using HPLC. The HPLC operating conditions were the same as those used for TCP quantification. Compounds that were possible products of the reaction were injected into the HPLC to identify the unknown product by matching the retention time and UV-VIS spectrum. The filtrate was also analyzed on Dionex 200li series liquid ion chromatograph to determine the concentration of organic acids that may have been formed.

Infrared analysis was carried out on the precipitate (in methanol) to get some indication of the functional groups on the reaction product. Infrared analysis was conducted on a Nicolet 510P FT-IR spectrometer manufactured by Nicolet (Madison, WI). A background subtraction was done to eliminate the effect of the methanol solvent. In addition, gas chromatograph/mass spectrometric (GC/MS) analysis of the reaction medium, as well as the precipitate dissolved in methanol, was done using a VG 70-25OHF mass spectrometer (VG Analytical, Manchester, England) in conjunction with a model 5790 Hewlett Packard GC. The GC/MS operating conditions were: column, DB-1 capillary thin film (60 m); He as carrier gas; column pressure 25 psi and split ratio 20:1; column and oven temperature 35°C for 2 min, then 7 C/min to 325°C; injector temperature, 260°C.

RESULTS AND DISCUSSION

Porphyrin-Catalyzed Oxidation of TCP

The optimum pH for Fe-diCl-Ph-catalyzed oxidation of TCP was determined to be pH 7.0. The reaction rate was reduced to 40% at pH 5.0,

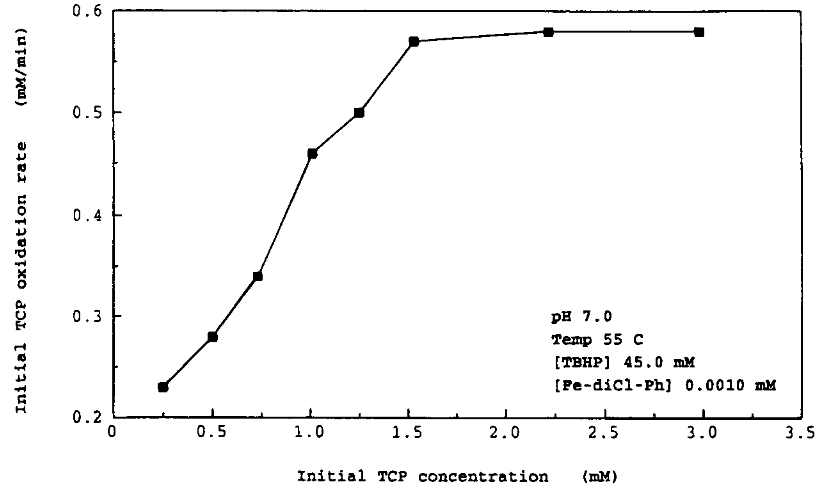

Fig. 2. Effect of initial TCP concentration on Fe-diCl-Ph-catalyzed TCP oxidation with TBHP.

but was reduced only to 80% at pH 9.0 compared to the rate at pH 7.0. All subsequent reactions with all three porphyrins were conducted at this pH.

Under assay conditions described above, complete conversion of TCP was observed with Fe-diCl-Ph and Fe-diF-Ph as catalyst. However, TCP conversion was incomplete (35%) in reactions catalyzed by Fe-pro-Ph. With Fe-pro-Ph, TCP conversion could be increased by increasing the initial porphyrin concentration, indicating that TCP conversion was reduced by ring-centered oxidation and inactivation of the porphyrin. Fe-diCl-Ph and Fe-diF-Ph apparently exhibited greater conversion because of greater stability to ring-centered oxidation.

Batch experiments, with Fe-pro-Ph, Fe-diCl-Ph, and Fe-diF-Ph, were conducted to study the effect of initial TCP concentration on TCP oxidation. In each case, the initial rate increased linearly with initial TCP concentration and then leveled off. Further increase in initial TCP concentration did not result in higher initial oxidation rates. With Fe-diCl-Ph (0.0010 m*M*) as catalyst, no further increase in reaction rate was seen at TCP concentrations above 1.5 m*M* (Fig. 2). This reaction exhibited saturation kinetics, a characteristic typical of enzyme-catalyzed reactions (such as ligninase-catalyzed reactions). For comparison, the initial rates of reaction for each system at an initial TCP concentration of 1.0 m*M* and porphyrin concentration of 0.0010 m*M* were: Fe-pro-Ph, 0.10 m*M*/min; Fe-diCl-Ph, 0.50 m*M*/min; and Fe-diF-Ph, 0.72 m*M*/min.

In addition to demonstrating that porphyrin-catalyzed oxidation reactions exhibit saturation kinetics, the initial rate vs initial TCP concentration data were used to show that stoichiometric amounts of TCP were not consumed for reaction mixtures containing the same amount of porphyrin. Table 1 shows in that reactions catalyzed by Fe-diCl-Ph the mmol of TCP

Table 1
Effect of Initial TCP Concentration on TCP Conversion[a]

Initial TCP mM	Final TCP mM	TCP Converted mM	mmoles TCP/mmole Fe-diCL-Ph
0.25	0.00	0.25	250
0.50	0.09	0.41	410
0.73	0.15	0.58	580
1.01	0.31	0.70	700
1.25	0.47	0.78	780
1.53	0.62	0.91	910
2.22	1.27	0.95	950
2.98	1.88	1.10	1100

[a] The Fe-diCl-Ph-catalyzed reaction was conducted at 55°C, pH 7.0. The Fe-diCl-Ph concentration was 0.0010 mM and TBHP concentration was 45.0 mM, the TCP concentration was caried from 0.20–3.00 mM.

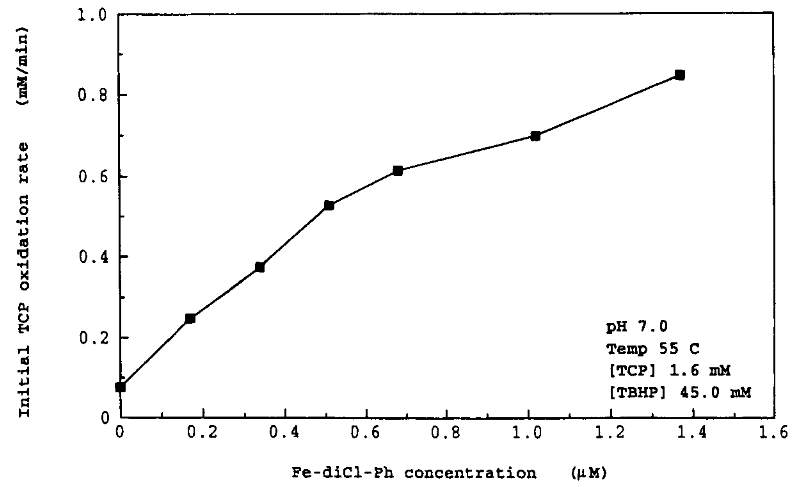

Fig. 3. Effect of Fe-diCl-Ph concentration on TCP oxidation rates. TBHP was the oxidizing agent.

oxidized/mmol of porphyrin is different in each run and increases with initial TCP concentration, thus supporting a catalytic role for porphyrins in the TCP oxidation reaction.

The effect of porphyrin concentration on TCP oxidation rate was also investigated. Porphyrin concentration was varied from 0.0–0.0014 mM. As expected, increasing the catalyst concentration in each case resulted in a roughly linear increase in the initial oxidation rates (Fig. 3).

The extent of dechlorination was determined for all three catalysts (Fe-pro-Ph, Fe-diF-Ph, and Fe-diCl-Ph) at temperatures of 55–100°C

(Table 2). Almost complete dechlorination (93.7%) of the oxidized fraction of initial TCP present occurred when Fe-pro-Ph was used as catalyst at a reaction temperature of 55°C. The extent of dechlorination by Fe-pro-Ph dropped to 75.3% at 100°C possibly because the higher temperature increased the rate of ring-centered oxidation. The ability to dechlorinate TCP was found to be lower in the case of both Fe-diCl-Ph and Fe-diF-Ph, however, the extent of dechlorination did not change significantly with increased reaction temperature, suggesting again that both these porphyrins were much more stable than Fe-pro-Ph with regard to ring-centered oxidation.

The filtrate from reaction mixtures was analyzed for organic acids by ion chromatography for organic acids. Very small amounts of formate, acetate, and propionate (all <0.01 mmol/mmol TCP) were detected. Infrared analysis of the precipitate indicated the presence of one or more carbonyl groups as indicated by a major absorbance at 1670 cm^{-1}. GC-MS analysis of the filtrate and precipitate led to the following observations:

1. The major products formed during TCP oxidation were the same irrespective of the choice of Fe-diF-Ph or Fe-diCl-Ph as the catalyst.
2. Except for one product, which had two chlorine atoms, isotopic analysis showed that all other chlorinated products contained only one chlorine atom, indicating that two of the three chlorine atoms had been removed from the TCP. This further confirms the data obtained from the chloride balance, which indicated that 60–70% dechlorination occurred in the Fe-diF-Ph or Fe-diCl-Ph catalyzed oxidation of TCP.
3. Based on the interpretation of the fragmentation patterns, the absence of aromatic compounds indicates that in addition to dechlorination, the benzene ring was also broken.

CONCLUSIONS

Three porphyrins, Fe^{3+}-protoporphyrin IX, Fe^{3+}-meso-tetra(*o*-dichlorophenyl) porphine sulfonate and Fe^{3+}-meso-tetra(*o*-difluorophenyl) porphine sulfonate have been shown to catalyze the oxidation 2, 4, 6-trichlorophenol. TCP conversion was incomplete in reactions catalyzed by Fe^{3+}-protoporphyrin IX owing presumably to ring-centered oxidation and inactivation. The two substituted porphyrins were observed to give 100% conversion of TCP under assay conditions used in these studies. Reaction rates were significantly greater for the substituted porphyrins. All three porphyrins exhibited saturation kinetics with TCP, as substrate and reaction rates increased linearly with porphyrin concentration.

Fe^{3+} protoporphyrin-catalyzed reactions were characterized by complete dechlorination of TCP oxidized, although TCP conversion was incomplete. All reaction products in reactions catalyzed by the substi-

Table 2
Dechlorination Activity of Porphyrin Catalysts[a]

Porphyrin	Temperature C	Porphyrin Conc mM	TCP Initial mM	TCP Final mM	TCP Converted mM	Measured CI mM	TCP Dechlorination[b] %
Fe-pro-Ph	55	0.0080	2.10	1.36	0.74	2.09	93.7
Fe-diCl-Ph	55	0.0080	2.11	0.00	2.11	4.00	63.3
Fe-diF-Ph	55	0.0080	2.13	0.00	2.13	4.00	62.5
Fe-pro-Ph	55	0.0330	1.92	0.45	1.48	3.61	81.3
Fe-diCl-Ph	55	0.0330	1.93	0.00	1.93	3.61	62.2
Fe-pro-Ph	100	0.0330	1.89	0.44	1.46	3.30	75.3
Fe-diCl-Ph	100	0.0330	1.90	0.00	1.90	4.11	72.1
Fe-diF-Ph	100	0.0330	1.90	0.00	1.90	4.11	72.1

[a] Batch reactions were conducted at two temperatures, 55 and 100°C, at pH 7.0, and two porphyrin concentrations, 0.0080 and 0.0330 mM. Reactions were carried out in 0.10M phosphate buffer at pH 7.0 and a TBHP concentration of 45.0 mM.

[b] Based on complete dechlorination of TCP converted, not an initial TCP present.

tuted porphyrins were at least partially dechlorinated. Most were monochlorinated compounds with no indication that the benzene ring had survived intact.

Porphyrin-catalyzed oxidation has been shown to be a potentially viable process concept for partial oxidation and detoxification of chlorinated phenols.

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