Pergamon

Tetrahedron Letters, Vol. 36, No. 42, pp. 7681-7684, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01611-2

Novel Oxidative Pathway of *para*-Substituted Phenols in Cytochrome P450 Chemical Model: Substituent Elimination Accompanying *ipso*-Substitution by the Oxygen Atom of the Active Species[1]

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Abstract: The *meso*-tetrakis(2,6-difluorophenyl)porphinatoiron(III) chloride-mCPBA system, a chemical model of cytochrome P450, catalyzed substituent elimination from various *para*-substituted phenols. An experiment using [^{18}O]mCPBA proved that these reactions are accompanied with *ipso*-substitution by the oxygen atom of the active species of the P450 model.

Cytochromes P450 (P450) are heme-containing monooxygenases that catalyze the oxidative metabolism of a wide variety of compounds.[2] Many metalloporphyrins have been synthesized in attempts to elucidate the molecular mechanisms of these enzymes and to develop practical catalysts for oxidative reactions.[3] We have employed a variety of metalloporphyrins as chemical models of P450 for studies on drug metabolism.[4-11] P450 models are able to provide large amounts of metabolites and unstable metabolites, and are also useful in analyzing metabolic pathways and mechanisms.

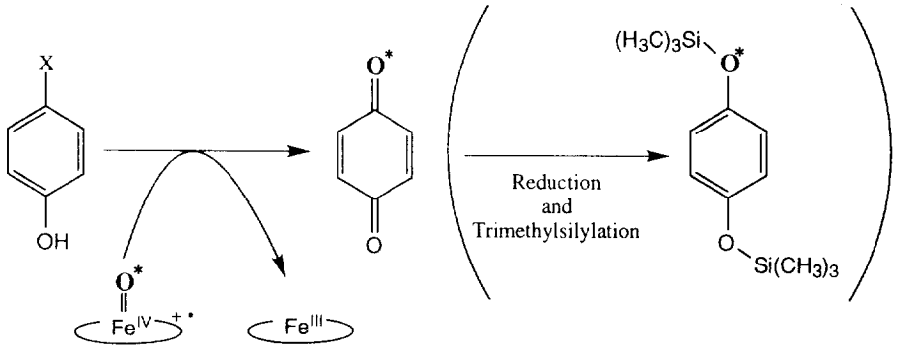

Fig. 1. Substituent eliminations from *p*-substituted phenols.
X=F, Cl, Br, NO_2, CN, CH_2OH, $COCH_3$,COPh, COOH
p-Benzoquinone was detected by GC-MS as trimethylsilylated hydroquinone.

The present study focuses on the P450-catalyzed conversion of *p*-substituted phenols. We have already reported that *p*-phenoxyphenol and *p*-methoxyphenol suffer "cleavage of the oxygen-aromatic ring bond" in P450 models and rat liver microsomes. It was also proved that this cleavage reaction occurs accompanied with *ipso*-substitution by the oxygen atom of the active species, and a hydroxy group of the substrate is

necessary for this pathway to operate.[10] On the basis of these results, we considered that similar reactions might generally occur in various *p*-substituted phenols other than *p*-hydroxyarylethers, resulting in the elimination of the substituents (Fig. 1). Dehalogenation of halogenated phenols is known as a P450-mediated metabolic process,[12-14] which also supports our hypothesis. The dehalogenation is novel in oxidation systems containing metalloporphyrins, and the elimination of other substitutuents is a completely new type of oxidative pathway.

A *meso*-tetrakis(2,6-difluorophenyl)porphinatoiron(III) chloride-mCPBA (Fe(III)TDFPPCl-mCPBA) system was employed as a P450 model in the present work. mCPBA contains preactivated oxygen and it immediately reacts with metalloporphyrin to produce the active species, which is analogous to that of P450. Various *p*-substituted phenols were employed as substrates (X=F, Cl, Br, NO_2, CN, CH_3, CH_2OH, $COCH_3$, COPh, COOH). mCPBA (final 10 mM) was added to a solution containing Fe(III)TDFPPCl (50 μM) and substrate (10 mM) in dichloromethane / methanol (9/1). The reaction mixture was stirred vigorously for 20 min at room temperature, then dichloromethane and methanol were evaporated by argon flushing. The residue was reduced with ascorbic acid in order to convert *p*-benzoquinone into hydroquinone and trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine. After removal of excess BSTFA by argon flushing, the residue was dissolved in a small amount of acetone and analyzed by GC-MS.[15]

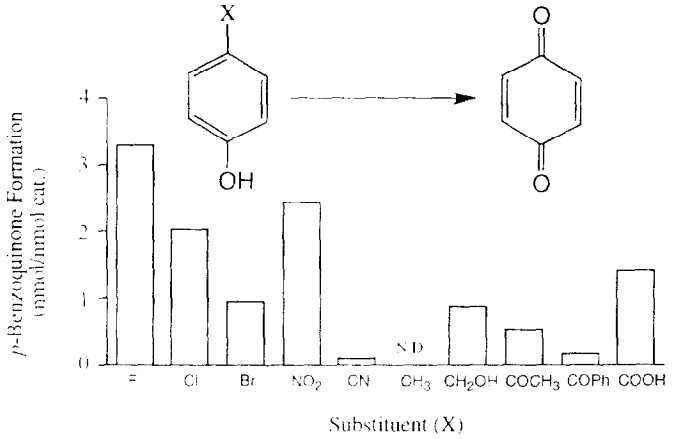

Fig. 2 Oxidation of *p*-substituted by Fe(III)TDFPPCl-mCPBA system.
p-Benzoquinone was quantified as trimethylsilylated hydroquinone.
N.D.; <0.05 nmol/nmol cat.

Fig. 2 shows the results for the substituent elimination from various *p*-substituted phenols to afford *p*-benzoquinone. *p*-Benzoquinone was quantified as trimethylsilylated hydroquinone. These results show that all substituents, except the methyl group, were eliminated and relatively large amounts of *p*-benzoquinone were formed when *p*-halogenophenol (F>Cl>Br), *p*-nitrophenol and *p*-hydroxybenzoic acid were used as substrates. The order of yields in *p*-halogenophenols corresponded to the size of halogeno groups. This result indicated that the active species is highly hindered in its approach to the *ipso*-position by a larger substituent such as Br. It is also interesting that this type of reaction occurred in the case of some substrates whose

substituents are attached to the aromatic ring through a carbon-carbon bond, such as *p*-hydroxyacetophenone, *p*-hydroxybenzyl alcohol, *p*-hydroxybenzoic acid, *etc.*, since the carbon-carbon bond, which is very stable and has generally been thought to resist oxidation, was cleaved. Carbon-carbon bond cleavage is known to be catalyzed by some P450 isozymes involved in the synthesis of steroid hormones from cholesterol in steroidogenic tissues, *i.e.* the adrenal cortex, testis and ovary.[16] However, it seems that the cleavage reaction in the present study is entirely different from such cases.

When Fe(III)TDFPPCl or mCPBA was omitted from the complete system, little formation of *p*-benzoquinone was detected, in all cases.

Table 1. ^{18}O Incorporation into *p*-Benzoquinone Formed by the Fe(III)TDFPPCl-[^{18}O]mCPBA System

Substituent (X)	F	Cl	Br	NO_2	CN	CH_2OH	$COCH_3$	COPh	COOH
^{18}O Incorporation	92	82	81	92	90	95	92	92	92

Note. *p*-Benzoquinone was detected as the trimethylsilylated hydroquinone form. The ^{18}O content was calculated from the 256/254 peak ratio in the mass spectra of the trimethylsilylated hydroquinone and then corrected on the basis of the original ^{18}O content of [^{18}O]mCPBA.

To elucidate the mechanism of these substituent elimination reactions by our P450 model, [^{18}O]mCPBA was utilized as an oxidant (mCPBA is the origin of oxygen in the active species of porphyrin). *p*-Benzoquinone was converted into trimethylsilylated hydroquinone in a similar manner to that described above and analyzed by GC-MS. The ^{18}O content in *p*-benzoquinone was calculated from the 256/254 (M^++2/M^+) peak ratio in the mass spectrum of trimethylsilylated hydroquinone and then corrected to take account of the original ^{18}O content of [^{18}O]mCPBA. The ^{18}O content in *p*-benzoquinone was more than 80% in every case (Table 1), showing that one oxygen of *p*-benzoquinone was derived from the oxygen atom of the active species on heme iron. That is, the substituent elimination reaction occurred accompanied with the replacement of the substituent group by the oxygen atom of the active species (*ipso*-substitution).

We assumed that the hydroxy group might be required for the *ipso*-substitution by anlogy with the previous work, namely *ipso*-substitution in *p*-hydroxyarylethers.[11] To confirm the requirement of the hydroxy group, we investigated P450 model-catalyzed oxidation of various *p*-substituted toluenes. Little *p*-cresol was detected when any *p*-substituted toluene was employed as the substrate (data not shown) and it was revealed that the hydroxy group at the *p*-position is required for all *ipso*-substitution. Therefore, a mechanism *via* formation of phenoxy radical was suggested, as in the previous work.[11] That is, one electron plus a proton is abstracted from the phenol moiety of *p*-substituted phenols or a hydrogen radical is abstracted from its hydroxy group by the iron-oxenoid to give a phenoxy radical. This radical delocalizes on the aromatic ring and is distributed to the *ipso*-position of the aromatic ring, followed by rebound of the hydroxyl-radical equivalent on the heme iron. This intermediate breaks down to afford *p*-benzoquinone and the substituent group is eliminated simultaneously. Further investigation into this mechanism is in progress.

In short, it was proved that various *p*-substituted phenols undergo substituent elimination reaction in our P450 model system. These reactions were accompanied with *ipso*-substitution by the oxygen atom on the active species of porphyrin.

The relevance of these results to metabolism *in vitro* and *in vivo* should be investigated. We have recently revealed that this type of reaction occurs in a rat liver microsomal system. Details of this new type of metabolism will be reported in another paper.

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(*Received in Japan* 25 *July* 1995; *revised* 23 *August* 1995; *accepted* 25 *August* 1995)