Pronounced Axial Thiolate Ligand Effect on the Reactivity of High-Valent Oxo−Iron Porphyrin Intermediate

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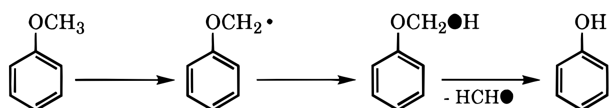
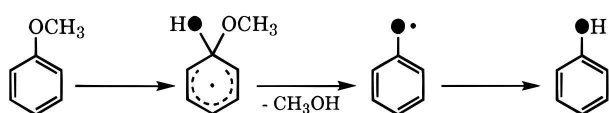
Among heme enzymes, cytochrome P450 has the strongest oxidizing ability, e.g. only P450 can hydroxylate nonactivated alkanes.[1] The distinctive structural features of P450 are the unusual thiolate coordination to the heme and also the extreme hydrophobicity of its active site,[1] and much interest has been focused on the effect of the axial thiolate ligand on the O−O bond cleavage step in the catalytic cycle of P450. Recently Gross and Nimri[2] have reported an axial ligand effect on styrene epoxidation mediated by iron porphyrin−ozone systems, but they employed halide anion or alkoxy anion as a ligand and did not use a thiolate or an imidazole ligand, which may be more relevant to heme-containing enzymes. In addition, little is known yet about the axial ligand effect on *the reactivity* of the oxidizing intermediate of the heme enzymes. We report here that a high-valent oxo−iron porphyrin intermediate with a thiolate ligand has similar reactivity to that of cytochrome P450.

Oxidative *O*-dealkylation of alkyl aryl ethers is one of the major metabolic reactions catalyzed by cytochrome P450.[1] There are two generally accepted mechanisms, that is, the H atom abstraction mechanism and the *ipso*-substitution mechanism.[3]

• H atom abstraction mechanism ($k_H/k_D > 6$)



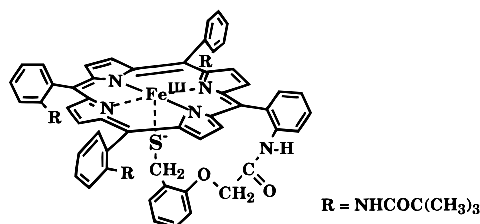
• *ipso*-substitution mechanism ($k_H/k_D \approx 1$)



Clear differences between these two mechanisms are observed in the kinetic isotope effects (KIEs) and in the origin of the oxygen atom of the resulting phenolic hydroxy group. (In the reaction scheme, the filled O indicates the oxygen atom originated from the active oxidizing intermediate.) So far, it is thought that the mechanism which actually operates depends on the oxidizing system used, namely in the cytochrome P450-dependent enzymatic reaction and the iron porphyrin−iodosylbenzene (PhIO) systems, the former mechanism operates, and in hydroxyl radical-mediated reactions, the latter does.[3c]

In a previous paper,[4] we have shown that the *O*-demethylation mechanisms of *p*-dimethoxybenzene can be used as a probe for differentiating the nature of some oxidative intermediates. Therefore, we examined the *O*-demethylation mechanisms of *p*-dimethoxybenzene in various iron porphyrin−oxidant systems, in addition to a microsomes from livers of a phenobarbital treated rats−NADPH/O_2 system[4] and an expressed human CYP1A2−NADPH/O_2 system. We used five kinds of *meso*-tetraaryl iron porphyrins. Among them, “Swan−Resting” form porphyrin (S.R. complex, Scheme 1), which was previously reported by us, is a unique complex which retains its axial thiolate coordination during catalytic oxidation reactions.[5]

Scheme 1. S.R. Complex



First of all, we investigated the modes of O−O bond cleavage mediated by these iron porphyrins by using peroxyphenylacetic acid (PPAA), which has frequently been used as a probe for this purpose.[5b,6] Though S.R. is an oxido-stable complex compared to other thiolate-ligated porphyrins,[7] the amount of each peroxy acid used in the reactions below was kept at 1 mol equiv to the iron porphyrins, because it is necessary to minimize the decomposition of the S.R. complex and the secondary oxidation of the formed acid by active species. In the other four porphyrin systems, the same amount of oxidant was used to get comparable results. In every iron porphyrin−PPAA system examined in either solvent, benzene or dichloromethane, phenylacetic acid was the major product, which indicated the predominance of heterolytic O−O bond cleavage and compound I formation.[8]

Next, the KIEs in the *O*-demethylation of *p*-dimethoxybenzene were examined (Table 1).[9,10] In the rat liver microsomes− and human CYP1A2−NADPH/O_2 systems, *p*-dimethoxybenzene was *O*-demethylated with high KIE values (>10). Among the porphyrin−oxidant systems, only the S.R.−PPAA system showed high KIE values. All other iron porphyrin−oxidant systems gave low KIE values (≈1.0). Further, we have investigated the ^{18}O incorporation from ^{18}O-enriched oxidants in the *O*-demethylation of *p*-dimethoxybenzene (Table 2), and again unambiguous results, which are in harmony with those in the KIE experiments, were obtained. In the rat liver microsomes−NADPH/O_2 system, *p*-dimethoxybenzene was

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(8) The following papers strongly support the formation of the two-electron oxidizing active intermediate in dichloromethane. (a) Groves, J. T.; Watanabe, Y. *J. Am. Chem. Soc.* **1988**, *110*, 8443. (b) Watanabe, Y.; Yamaguchi, K.; Morishima, I.; Takehira, K.; Shimizu, M.; Hayakawa, T.; Orita, H. *Inorg. Chem.* **1991**, *30*, 2582.

(9) Abbreviations used: TPP, *meso*-tetraphenylporphyrin; TMP, *meso*-tetramesitylporphyrin; TPFPP, *meso*-tetrakis(pentafluorophenyl)porphyrin; 1-MeIm, 1-Methylimidazole.

(10) Since the amount of peroxy acid used in the reactions with porphyrins was kept at 1 mol equiv to the iron porphyrins, the yields of *p*-methoxyphenol were relatively low (ranging from 18 to 80 μM). The turnover value in the reaction with rat liver microsomes was 16. A small amount of 2,5-dimethoxyphenol, the aromatic hydroxylation product, was also detected in every system.

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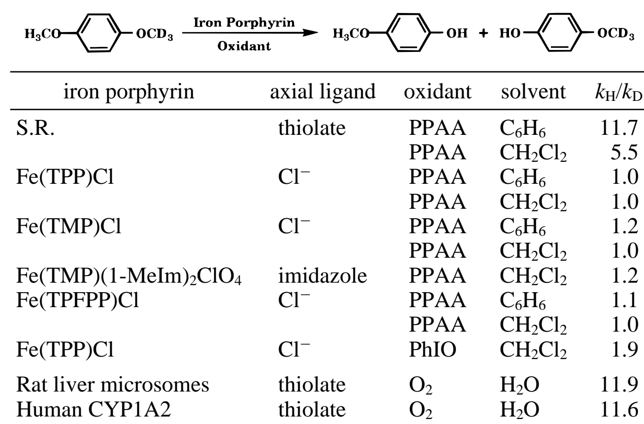
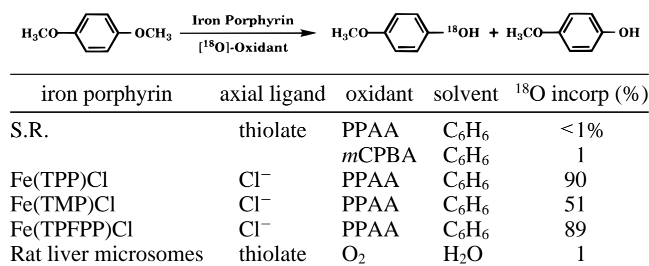
Table 1. Kinetic Isotope Effects on the *O*-Demethylation of *p*-Dimethoxybenzene by Various Iron Porphyrin−Oxidant Systems, As Well As Rat Liver Microsomes−NADPH/O_2 and Human CYP1A2−NADPH/O_2 Systems



iron porphyrin	axial ligand	oxidant	solvent	k_H/k_D
S.R.	thiolate	PPAA	C_6H_6	11.7
		PPAA	CH_2Cl_2	5.5
Fe(TPP)Cl	Cl^-	PPAA	C_6H_6	1.0
		PPAA	CH_2Cl_2	1.0
Fe(TMP)Cl	Cl^-	PPAA	C_6H_6	1.2
		PPAA	CH_2Cl_2	1.0
$Fe(TMP)(1\text{-}MeIm)_2ClO_4$	imidazole	PPAA	CH_2Cl_2	1.2
Fe(TPFPP)Cl	Cl^-	PPAA	C_6H_6	1.1
		PPAA	CH_2Cl_2	1.0
Fe(TPP)Cl	Cl^-	PhIO	CH_2Cl_2	1.9
Rat liver microsomes	thiolate	O_2	H_2O	11.9
Human CYP1A2	thiolate	O_2	H_2O	11.6

[a] The reactions with porphyrins were carried out at room temperature under argon for 10 min. [Fe(Por)] = 1.0 mM; [PPAA] = 1.0 mM; [substrate] = 0.10 M. The reaction with rat liver microsomes or human CYP1A2 was carried out at 37 °C under air for 10 min. Rat liver microsomes were prepared according to ref 4. Human CYP1A2 was purchased from GENTEST Corp. Kinetic isotope effects were determined by GC/SIM from the M^+ peak area ratio of the products.

Table 2. ^{18}O Incorporations from ^{18}O-Enriched Oxidants in the *O*-Demethylation of *p*-Dimethoxybenzene by Various Iron Porphyrin−Oxidant Systems and the Rat Liver Microsomes−NADPH/O_2 System



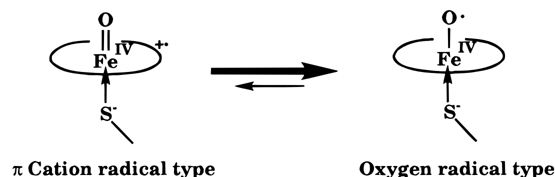
iron porphyrin	axial ligand	oxidant	solvent	^{18}O incorp (%)
S.R.	thiolate	PPAA	C_6H_6	<1%
		*m*CPBA	C_6H_6	1
Fe(TPP)Cl	Cl^-	PPAA	C_6H_6	90
Fe(TMP)Cl	Cl^-	PPAA	C_6H_6	51
Fe(TPFPP)Cl	Cl^-	PPAA	C_6H_6	89
Rat liver microsomes	thiolate	O_2	H_2O	1

[a] The reaction conditions were identical to those described in Table 1, except that ^{18}O-enriched oxidants were used. ^{18}O incorporations were determined by GC/SIM from the M^+ peak area ratio.

O-demethylated with low ^{18}O incorporation. Among the porphyrin−oxidant systems, such low ^{18}O incorporations were observed only in the S.R.−PPAA and S.R.−*m*-chloroperoxybenzoic acid (*m*CPBA) systems. In other iron porphyrin−oxidant systems, high ^{18}O incorporation values were obtained.

From the results above, it is clear that in the thiolate-ligated heme-containing systems, i.e., S.R. and P450 systems, *p*-dimethoxybenzene was *O*-demethylated with high KIE values and with low ^{18}O incorporation, which showed that the reaction proceeded by the H atom abstraction mechanism. In other iron porphyrin−oxidant systems with a chloride anion or an imidazole ligand, low KIE values and high ^{18}O incorporations were observed, which showed that the reaction proceeded in the *ipso*-substitution manner. The thiolate ligand has been believed to affect the O−O bond cleavage step in the catalytic cycle of P450. However, considering that in every porphyrin system PAA was the major product from PPAA, our data in this paper provide evidence that the two-electron oxidizing iron−oxo porphyrin intermediate of thiolate-ligated porphyrin has different reactivity to that of chloride anion- or imidazole-ligated porphyrin. Champion[11] has suggested on the basis of the resonance Raman spectroscopy that the iron−oxo π cation complex is not preferred, but rather the oxygen radical configuration is favored as an oxidative active intermediate of P450s (Scheme 2), because of the strong π-electron-donating effects of thiolate ligand. On the other hand, in the histidine-ligated heme proteins, the iron−oxo π cation radical intermediate is favored. Champion speculated that the difference of reactivity between P450 and peroxidases might arise from the isoelectronic states of the oxidative intermediates. The results presented in this paper are the first experimental evidence to support this speculation. Furthermore, it is suggested that the oxygen radical type intermediate can abstract the hydrogen atom from the substrate more easily than the iron−oxo π cation radical intermediate.

Scheme 2. Equilibrium between Two Isoelectronic Iron−Oxo Structures Involving Thiolate Ligated Heme



In conclusion, we have established that the thiolate ligand has a marked influence on the reactivity of the high-valent iron−oxo porphyrin intermediate. The high-valent iron−oxo intermediate of thiolate-ligated porphyrin, such as P450s and S.R., has a stronger hydrogen-atom-abstracting ability than that of chloride anion- or imidazole-ligated porphyrins. These findings imply that the preferred structure of the active oxidizing intermediate of thiolate-ligated porphyrins is of the oxygen radical type. Thiolate ligation plays an important role in the characteristic oxidizing ability of P450s, and thus, our findings are of great significance for both chemical and biological studies of P450s.

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