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Original Article

Oxidative Activation and Degradation of Organophosphorus Pesticides Mediated by Iron Porphyrins

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Biomimetic oxidation mediated by three types of iron porphyrins was examined for five organophosphrus pesticides, fenitrothion (**I**), cyanophos (**II**), tolclofos-methyl (**III**), butamifos (**IV**) and fenthion (**V**). The major products from **I**–**IV** were the corresponding phenols and each oxon formed *via* a phosphoxathiirane intermediate by oxidation at the P=S moiety similarly as reported for their mammalian metabolism by cytochrome P450 enzymes. Stepwise oxidation of the methylthio sulfur *via* sulfoxide to sulfone, and ester cleavage and oxidative desulfuration primarily proceeded for **V**. Both the electron distribution of the highest occupied molecular orbital and its energy level of the pesticides calculated by MNDO-PM3 were shown to control the reaction site and rate of oxidation at sulfur atoms. © Pesticide Science Society of Japan

Keywords: oxidation mediated by iron porphyrin, organophosphorus pesticides, MNDO-PM3 calculations.

INTRODUCTION

Oxidative metabolism catalyzed by cytochrome P450 enzymes (P450) is one of the most important degradative pathways for xenobiotics including pesticides. Many types of reactions such as hydroxylation of alkyl and aromatic carbons, dealkylation *via* stepwise oxidation, epoxidation of alkene and oxidation at nitrogen and sulfur atoms are known to be catalyzed.[1–3] The atomic oxygen originating from molecular oxygen is bound to iron of the protoporphyrin IX moiety in P450 and its transfer to a substrate proceeds in the presence of NADPH.

The Fe (valence, IV; spin state, S=1) porphyrin-oxygen cation radical has been strongly suggested as the prosthetic moiety in an active state by extensive theoretical studies and the triplet oxene is considered to be the most appropriate model.[4] In order to examine the mode of action of P450-catalyzed oxidation at a molecular level, Keserü *et al.* have applied computational methods including molecular orbital (MO) calculations and molecular dynamic simulations to the carbofuran-P450cam complex utilizing the three-dimensional structure of the enzyme.[5] Either hydrogen bonding or steric constraint imposed by the polypeptide backbone was found to highly control the oxidation of carbofuran near the active site and the major metabolic hydroxylation at the 3-position was correctly elucidated. Another approach has been to use synthetic metalloporphyrins as a model of P450 especially in the field of drug metabolism.[3,6,7] The various types of electron-deficient iron porphyrins have been recently synthesized to increase oxidative reactivity and moreover, the introduction of bulky phenyl groups at *meso*-positions of the porphyrin ring has successfully hindered the unfavorable formation of the μ-oxo dimer in the catalytic oxidation.[8] The co-existence of an oxidant such as hydrogen peroxide in organic solvent was found to well simulate the P450-catalyzed reactions of many chemicals including several carbamate insecticides.[3,9]

The oxidative desulfuration of phosphorothioate pesticides to form the corresponding oxon or *S*-oxidation of the alkylthio group to sulfoxide and sulfone is a well-known metabolic reaction catalyzed by P450.[10] These reactions have been extensively examined for the various radiolabels of parathion, and phosphoxathiirane is proposed to be a key intermediate which is activated to the oxon with a release of an elemental sulfur or reacts with water to form the corresponding phenol.[11] Oxidative desulfuration through this intermediate was found to be feasible in a theoretical analysis using a reaction coordinate method in MO calculations of model compounds.[12] In order to examine the applicability of synthetic iron porphyrins as a biomimetic model of P450, we carried out the oxidation of several organophosphorus pesticides using three types of iron porphyrins. The reactivity and product distribution were also examined with the aid of MNDO-

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PM3 calculations.

MATERIALS AND METHODS

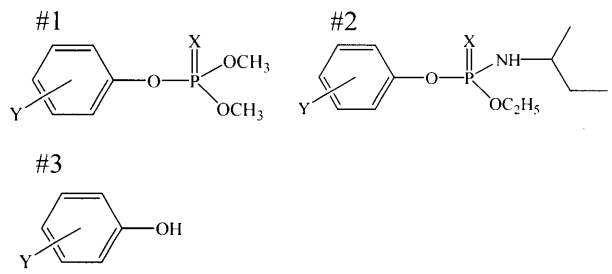
1. Chemicals

The chemical structures of fenitrothion (**I**) [*O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate], cyanophos (**II**) [*O*,*O*-dimethyl *O*-(4-cyanophenyl) phosphorothioate], tolclofos-methyl (**III**) [*O*,*O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate], butamifos (**IV**) [*O*-ethyl *O*-(5-methyl-2-nitrophenyl) *sec*-butylphosphoramidothioate], and fenthion (**V**) [*O*,*O*-dimethyl *O*-(3-methyl-4-methylthiophenyl) phosphorothioate] are listed in Table 1. **I**–**IV** uniformly labeled with ^{14}C at each phenyl ring were synthesized in our laboratory from corresponding ^{14}C-labeled phenols according to reported methods.[13–15] **V** uniformly labeled with ^{14}C at the phenyl ring was purchased from American Radiolabeled Chemicals, Inc. (St. Louis). The specific activities and radiochemical purities were 6.6 MBq/mg and 95.1% (**I**), 8.2 MBq/mg and 99.1% (**II**), 8.3 MBq/mg and 99.6% (**III**), 6.3 MBq/mg and 99.2% (**IV**), and 6.7 MBq/mg and 99.9% (**V**), respectively. The non-labeled pesticides except **V** and their potential metabolites (>95%) as listed in Table 1 were also synthesized in our laboratory according to reported methods.[16–18] **V** was purchased from Wako Pure Chemical Industries, Ltd. (Osaka) and other reagents were of the purest grade commercially available.

Table 1. Chemical structures of pesticides and their degradates

Compound	#[a]	X	Y	R.t.[b]
I	1	S	3-CH_3-4-NO_2	34.6
Ia	1	O	3-CH_3-4-NO_2	24.9
Ib	3	-	3-CH_3-4-NO_2	23.1
II	1	S	4-CN	31.4
IIa	1	O	4-CN	20.7
IIb	3	-	4-CN	17.9
III	1	S	2,6-Cl_2-4-CH_3	39.5
IIIa	1	O	2,6-Cl_2-4-CH_3	30.9
IIIb	3	-	2,6-Cl_2-4-CH_3	29.7
IV	2	S	5-CH_3-2-NO_2	40.1
IVa	2	O	5-CH_3-2-NO_2	31.0
IVb	3	-	5-CH_3-2-NO_2	29.3
V	1	S	3-CH_3-4-SCH_3	38.2
Va	1	S	3-CH_3-4-$SOCH_3$	24.9
Vb	1	S	3-CH_3-4-SO_2CH_3	29.2
Vc	3	-	3-CH_3-4-$SOCH_3$	12.0
Vd	3	-	3-CH_3-4-SO_2CH_3	15.8
Ve	1	O	3-CH_3-4-$SOCH_3$	16.0

[a] The basic chemical structure.



[b] Typical HPLC retention time obtained under the same conditions.

The chemical structures of the three porphyrins are shown in Fig. 1.

meso-Tetrakis(pentafluorophenyl)porphyrin iron (III) chloride [Fe(TPPF$_{20}$)] was obtained from Aldrich Chemical Co. (Milwaukee). *meso*-Tetrakis(2,6-dichlorophenyl)porphyrin iron (III) chloride [Fe(TPPCl$_8$)] was synthesized from Zn(TPPCl$_8$) as reported.[19] *meso*-Tetrakis(2,6-dichlorophenyl)-β-octabromoporphyrin iron (III) chloride [Fe(TPPCl$_8\beta$-Br$_8$)] was obtained by treating Zn(TPPCl$_8$) with *N*-bromoacetamide followed by metal exchange according as described previously.[20] These porphyrins were purified by an alumina column chromatography using chloroform.

2. Spectroscopies

Radioactivity in liquid samples was determined by mixing each aliquot with 10 mL of Packard Scintillator Plus® and analyzed by liquid scintillation counting (LSC) with Packard Model 1600TR and 2000CA spectrometers equipped with an automatic external standard. The background level of radioactivity in LSC was on average 30 dpm which was subtracted from the dpm value of a measured sample. Ultraviolet and visible absorption spectra were recorded using a Shimadzu UV-2550 spectrophotometer equipped with a thermo-

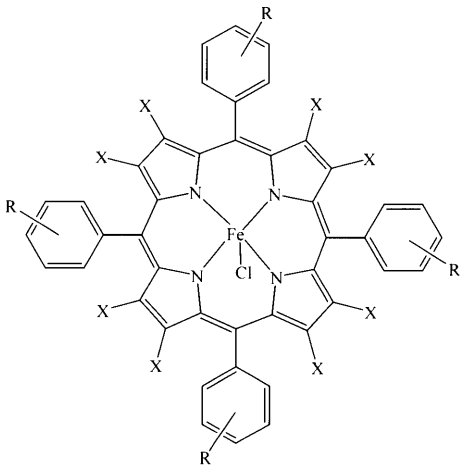


Fig. 1. Chemical structures of iron porphyrins used in this study. X=H, R=2,3,4,5,6-F_5 [Fe(TPPF$_{20}$)]; X=H, R=2,6-Cl_2 [Fe(TPPCl$_8$)]; X=Br, R=2,6-Cl_2 [Fe(TPPCl$_8\beta$-Br$_8$)].

stated cell holder.

3. Chromatographies

High-performance liquid chromatography (HPLC) was conducted using a Hitachi L-6200 Pump linked in series with an L-4000 UV detector and Packard Flow-one/Beta A-120 radio detector equipped with a 500-μL liquid cell in which Ultima-Flo AP® (Packard) was utilized as a scintillator. A Sumipax ODS A-212 column (150 mm×6 mm i.d. 5 μm, Sumika Chemical Analysis Service Ltd.) was employed at a flow rate of 1 ml min^{-1}. The following solvent program in a linear gradient mode was typically used; 0.01% trifluoroacetic acid (Solvent A) and acetonitrile (Solvent B): 0 min, %A–%B, 90–10; 40 min, %A–%B, 10–90; 40.1 min, %A–%B, 0–100; 60 min, %A–%B, 0–100. Thin-layer chromatography (TLC) was conducted using silica gel 60 F_{254} thin-layer chromatoplates (20×20 cm, 0.25-mm thickness, E. Merck) with a solvent system of toluene/ethyl formate/formic acid, 5/7/1 (v/v/v). The non-radiolabelled reference standards were detected by exposing TLC plates to ultraviolet light. Autoradiograms were prepared by exposing TLC plates to a BAS-IIIs Fuji Imaging Plate for several hours. The radioactivity on an imaging plate was detected using a Fuji Bio-Imaging Analyzer BAS-1500.

4. Porphyrin-Mediated Oxidative Reactions

Organophosphorus pesticides and porphyrin were all dissolved in 500 μl of chloroform/methanol (1/1, v/v). Regarding **I**–**IV**, kinetic profiles with their degradation pathways were determined for pesticide and porphyrin concentrations at 50 ppm ($1.5–2.0\times10^{-4}$ M) and 200 ppm ($1.2–2.0\times10^{-4}$ M), respectively. In the case of **V**, the degradation profiles were monitored under the same conditions, while kinetic data were obtained at a porphyrin concentration of 2 ppm (1.4×10^{-6} M) instead. The oxidative reaction was initiated by addition of 50 μl of hydrogen peroxide (*ca.* 0.8 M) under stirring at room temperature. A 50-μl aliquot of the reaction mixture was periodically taken with a microsyringe for direct HPLC and LSC analyses. The chemical identity of each degradate was confirmed by HPLC and TLC co-chromatographies with non-radiolabelled reference standards. The reaction of each pesticide in the presence of each porphyrin was conducted in duplicate for estimating the first-order degradation rate constant.

Since the oxidative desulfuration of an organophosphorus pesticide is known to result in the release of elemental sulfur, it was quantified by performing an isocratic HPLC analysis of the reaction mixture with the ODS A-212 column at 254 nm according to the method developed by Mcguire and Hamers.[21] The mobile phase was methanol/water (95/5, v/v) delivered at a flow rate of 1 ml min^{-1} and the typical retention time of elemental sulfur was 16.5 min. The detection limit was 5 ppm in this study.

Furthermore, the oxidative degradation of porphyrin by hydrogen peroxide was spectrophotometrically examined using Fe(TPPF_{20}). The porphyrin in 2.5 ml of chloroform/methanol (1/1, v/v) at a concentration of 5 ppm was added to a quartz glass cell (1-cm pathlength). The UV-vis spectra were periodically monitored for up to 40 min after adding 10 μl of hydrogen peroxide. The amount of porphyrin was determined from the absorbance at 405.5 nm (Soret band).

5. Computational Details

In order to examine the different reactivities between pesticides and porphyrins at a molecular level, the molecular geometries of each pesticide in lower energy states were first estimated computationally. For MO calculations of each pesticide, the initial geometry was derived from the standard values of bond lengths and angles. The torsional angles determining the orientation of *O*-alkyl, *O*-aryl and *N*-alkyl groups attached to the central phosphorus were initially adjusted to those of *O*,*O*-dimethyl *O*-phenyl phosphorothioate.[22] All of the bond lengths, bond angles and torsional angles were then fully optimized by the MNDO-PM3 calculations with the EF routine,[23] using the WINMOPAC program (version 2.0, Fujitsu Ltd.). In the case of **IV**, an *S*-configuration around the phosphorus was assumed, because the chemical reactivity of **IV** was considered unlikely to change with optical isomerism at the phosphorus according to the stereospecific oxidation of the structural analogue.[24]

6. Calculation of Degradation Rate Constant

The degradation rate constant (k) of each pesticide was estimated by applying the least-squares approximation method to the equation $P/P_0=\exp(-kt)$, where P and P_0 are concentrations of pesticide at time t (in hr) and $t=0$, respectively. The regression analyses including the non-linear one for estimating the relationship between k and molecular parameters of each pesticide such as the energy level of the molecular orbital and reaction indices calculated from electron distributions of frontier orbitals were conducted using the SigmaPlot 2000 program (SPSS Inc.).

RESULTS

1. Porphyrin-Mediated Oxidation of Pesticides

Radioanalysis of reaction mixtures showed that the recovery of ^{14}C was 94.6–105.6% of the applied dose and therefore, any loss of ^{14}C during the reaction was unlikely. Based on the direct HPLC analysis of the reaction mixtures, the first-order degradation rate constants for **I**–**V** in the presence of each porphyrin were estimated as listed in Table 2. The correlation coefficients were mostly greater than 0.9. The fastest degradation was observed for **II** in the presence of Fe(TPPF_{20}), while **IV** and **V** were found to be most susceptible to oxidative degradation when Fe(TPPCl_8) and Fe(TPP$Cl_8\beta$-Br_8) were used, respectively. The degradation rates were mostly insensitive to the porphyrin structure for **I** and **V**, but greater degradation of porphyrins Fe(TPPF_{20}) and Fe(TPPCl_8) than Fe(TPP$Cl_8\beta$-Br_8) was observed for the other pesticides.

Table 2. First-order degradation rate constants

Pesticide	Rate constant (k in hr^{-1})		
	Fe(TPPF$_{20}$)	Fe(TPPCl$_8$)	Fe(TPPCl$_8$$\beta$-Br$_8$)
I	0.0611	0.0433	0.0523
II	0.2977	0.0642	0.0395
III	0.1230	0.1260	0.0303
IV	0.1690	0.2040	0.0144
V	0.1236	0.1290	0.1339

The degradation profiles were found to be common to **I–IV**, irrespective of the porphyrins used. In most cases, the corresponding phenol of each pesticide was dominantly formed as compared with the oxon, as summarized in Table 3. These degradates were identified by HPLC and TLC co-chromatography with the authentic standards. Since the absence of porphyrin or hydrogen peroxide caused no degradation of pesticides, both species were indispensable for the oxidative degradation. After a reaction for 1 day, 26.5–60.0% of the applied ^{14}C was transformed to phenols in the presence of Fe(TPPF$_{20}$) and Fe(TPPCl$_8$). For the reactions of **II** and **IV** with Fe(TPPCl$_8$)/H_2O_2, a faster formation within 5–7 hr was observed and more emphasized for **II** using Fe(TPPF$_{20}$). When Fe(TPPCl$_8$$\beta$-Br$_8$) was used, less formation of the phenol (5.1–16.1%) was detected after 1 day. The amount of oxon of **I–III** was almost equal to or more than half of that of the phenol, but the opposite trend was observed for **IV** with

Table 3. Product distribution in porphyrin-mediated oxidation of **I–IV**

Compound	% of the applied ^{14}C[a]		
	Fe(TPPF$_{20}$)	Fe(TPPCl$_8$)	Fe(TPPCl$_8$$\beta$-Br$_8$)
I	15.4	31.6	72.5
Ia	21.1	18.7	8.9
Ib	60.0	47.5	16.1
II	39.9 (3)	59.4 (7)	82.5
IIa	17.7	14.1	6.5
IIb	41.5	26.5	11.0
III	0.0	0.0	73.9
IIIa	18.0	22.0	4.4
IIIb	43.2	44.5	8.3
IV	26.2	30.6 (5)	82.7
IVa	20.0	21.5	12.2
IVb	53.8	48.0	5.1

[a] After 1 day except for the experiments with values (hr) in parentheses.

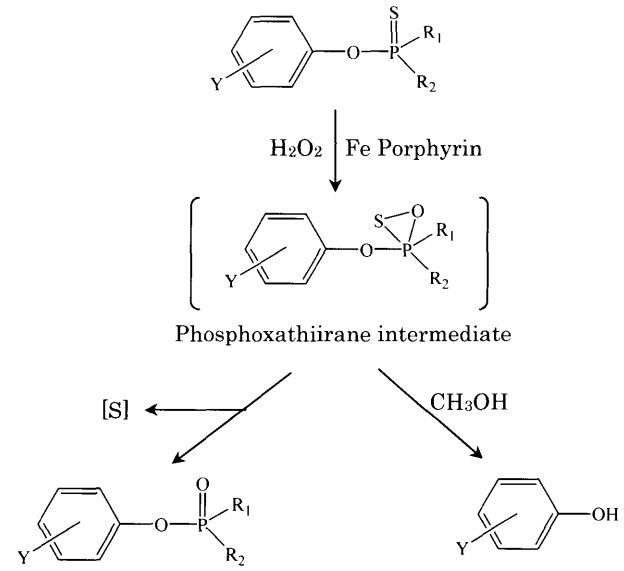


Fig. 2. Oxidative activation and degradation pathway of **I–IV** mediated by iron porphyrin. $R_1=R_2=OCH_3$ (**I–III**); $R_1=NHCH(CH_3)$-CH_2CH_3, $R_2=OC_2H_5$ (**IV**).

Fe(TPPCl$_8$$\beta$-Br$_8$). These results showed that the two degradation pathways were predominant for **I–IV** in the porphyrin-mediated oxidation, one of which was oxidative cleavage of the P–O-aryl bond and the other was oxidative desulfuration (Fig. 2). There are 10–20 minor degradates being detected by HPLC but none of them amounted to greater than 2% of the applied ^{14}C. The stepwise oxidation of the aryl methyl group was considered to proceed for **I**, **III** and **IV**, but the peaks corresponding to CH_2OH- and COOH-derivatives of each pesticide could not be detected by HPLC (data are not shown).

When Fe(TPPF$_{20}$) and Fe(TPPCl$_8$) were used, 40.6–81.1% of the parent pesticides were converted to the two main degradates. The elemental sulfur possibly released *via* oxidative desulfuration could not be detected in any reaction mixtures. In most cases, 10–20% of the pesticide was converted to the oxon and therefore, the elemental sulfur with a concentration of >5–10 ppm should have been detected. Since the elemental sulfur has been reported to be very reactive and found to attach to the apoprotein of P450,[11] it was most likely to react with the porphyrin molecule. The oxidation reaction mostly stopped after 1 day with a significant amount of unchanged parent pesticide remaining in some cases, typically the reactions mediated by Fe(TPPCl$_8$$\beta$-Br$_8$). Spectrophotometric monitoring of the reaction of Fe(TPPF$_{20}$) with hydrogen peroxide showed a rapid change in the UV-vis spectrum of the porphyrin with a half-life of 18.1 min. Oxidative decomposition has been reported for rat liver microsomal P450 and several water-soluble propentdyopents and maleimides were detected as degradates.[25] Therefore, the reactive porphyrin

species which failed to react with the pesticide molecule was likely to be further destroyed by hydrogen peroxide. These degradative processes for porphyrins would result in the non-catalytic oxidation of pesticides.

The oxidation profiles of **V** were found to be much different from those of **I**–**IV** and examined in more detail by using Fe(TPPF$_{20}$), as shown in Fig. 3. Most of **V** disappeared even after 1 hr and the main degradate was **Va** (40.1%) with a smaller amount of **Vb** (4.5%) formed *via* successive oxidation of the methylthio group at the 4-position of the phenyl ring. At the same time, **Vc** and **Vd** amounted to 23.7% and 23.2% of the applied ^{14}C, respectively, but no trace of 3-methyl-(4-methylthio)phenol or the oxon derivative of **V** was detected by HPLC. Further reaction resulted in the formation of more **Ve** *via* desulfuration of **Va**. The amount of **Vd** gradually increased with a concomitant decrease of **Vc** through oxidation. The good ^{14}C recovery showed insignificant mineralization of the phenyl ring in this system. Based on these results, **V** was most likely to primarily undergo oxidation of the thiomethyl group followed by oxidative desulfuration and cleavage of the P–O-aryl linkage *via* phosphoxathiirane intermediate, as proposed in Fig. 4.

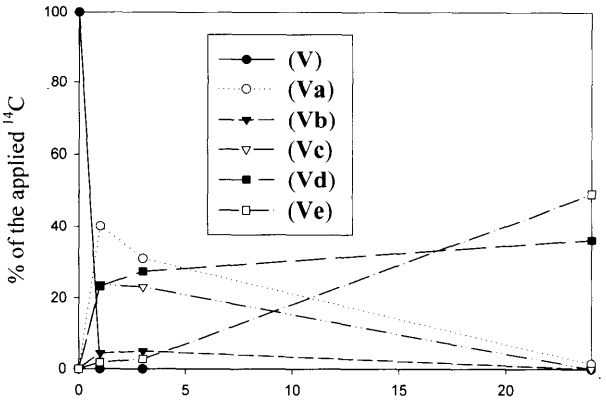


Fig. 3. Oxidative activation and degradation of **V** by Fe(TPPF$_{20}$) in the presence of H_2O_2.

2. *MO Calculations*

The geometrical and electronic parameters of **I**–**V** estimated by MNDO-PM3 calculations are listed in Table 4. The orientation of the three substituents attached to the central phosphorus was almost the same for **I**–**III** and **V** where the aryloxy moieties and one of the methoxy groups were located in the *gauche* position relative to the P=S moiety with torsional angles of −50~−60 deg. The other methoxy groups were in the *trans* position. In the case of **IV**, the bulkiness of the $NHCH(CH_3)CH_2CH_3$ group made the methoxy group separate from the *gauche* orientation toward the *trans* one. The partial charges on the sulfur of the P=S moiety were not significantly different among **II**, **III** and **V** and a slight shift to positive was observed for **I** and **IV**. The positive charge on phosphorus slightly decreased for **IV** by coordination of nitrogen but was almost the same for the other pesticides. The charges on these atoms seemed not to have a meaningful correlation with the degradation rates. The bond order of the P=S bond was also found to be insignificantly changed with the molecular structure of the pesticides.

Since the active oxygen species attached to the iron of the porphyrin is considered to be electron-deficient,[26] the f_E index was calculated with the following equation for the highest occupied MO (HOMO).

$$f_E = 2\Sigma C^2_{HOMO,i} \ (i = s, p_x, p_y, p_z)$$

$C_{HOMO,i}$ means the coefficient of each atomic orbital (s, p_x, p_y and p_z) constructing the HOMO. Since the P=S and C–S sulfur atoms are most likely to be the primary reaction site of the pesticides, their f_E indices were calculated as listed in Table 4 together with the energy level of HOMO. About half (*ca.* 45%) of the lowest unoccupied MO was found to be distributed at the P=S sulfur atom in any pesticide judging from the electron distribution estimated by summing the square of coefficient of each atomic orbital. The HOMO most likely to participate in the oxidation reaction was located at the energy level of −8.513~−9.783 eV. About 70–80% of HOMO was distributed at the P=S sulfur of **I** and **IV** and much less for **II** (44.4%) and **III** (17.4%), based on a similar calculation of the coefficient of each atomic orbital as above. In contrast, about half of the electrons in the HOMO were located at the C–S sulfur for **V**, indicating more reactivity at this site. The electrons mostly distributing at the P=S sulfur were found in

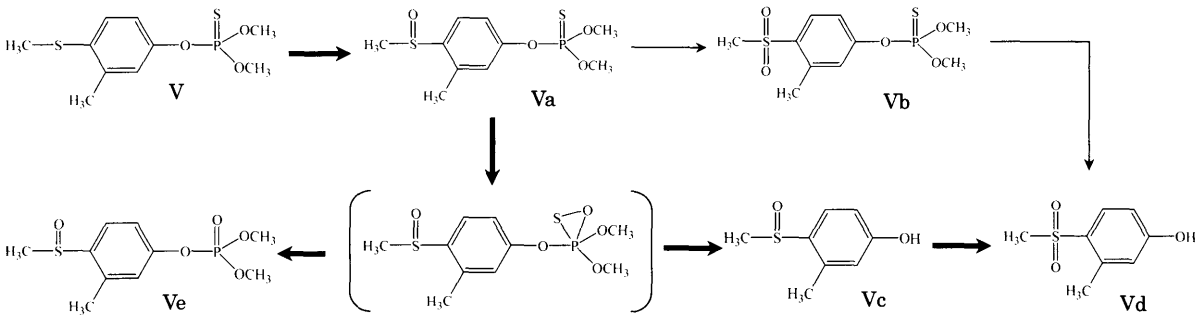


Fig. 4. Oxidative activation and degradation pathway of **V** mediated by iron porphyrin.

Table 4. MNDO-PM3 calculations for **I**–**V**

	I	**II**	**III**	**IV**	**V**
Torsional angle (deg)					
<SPOC (aryl)	−62.5	−62.5	−60.0	63.2	−58.7
<SPOC (methyl)	−50.3	−50.3	−50.6	142.0	−52.0
<SPOC (methyl) or <SPNC (alkyl)	−178.0	−178.0	−179.3	176.1	179.7
Partial charge					
P	+1.84	+1.83	+1.82	+1.73	+1.83
S (P=S)	−0.56	−0.66	−0.67	−0.63	−0.67
S (C–S)	—	—	—	—	+0.089
Bond order, P=S	1.139	1.134	1.129	1.157	1.128
HOMO E_L [a]	−9.783	−9.555	−9.175	−9.572	−8.513
f_E [b]	1.447	0.887	0.348	1.591	0.038

[a] HOMO, highest occupied molecular orbital; E_L, energy level of HOMO in eV. [b] Reaction index at the P=S sulfur defined as $2\times S\ Ci^2$. Ci means the coefficient of each atomic orbital in HOMO. In the case of **V**, f_E at the C–S sulfur was estimated to be 1.014.

lower-energy MOs instead of HOMO; HOMO+1 (**II**, 91% at −9.744 eV; **V**, 88% at −9.522 eV) and HOMO+3 (**III**, 75% at −9.657 eV). The f_E index alone could not explain the relative order of the first-order rate constants among the five pesticides. However, the favorable distribution of HOMO at the P=S sulfur for **I**–**IV** but at the C–S sulfur for **V** was most likely to correspond to the different reaction profiles, that is oxidative desulfuration and successive *S*-oxidation.

DISCUSSION

The usage of the three synthetic iron porphyrins in the presence of hydrogen peroxide as an oxidant could demonstrate the oxidative desulfuration and *S*-oxidation of organophosphorus pesticides being catalyzed by P450. However, there were many differences in the reaction profiles, that is much less turnover, more feasible cleavage of the P–O-aryl linkage, and no formation of C-hydroxylated derivatives being observed for their mammalian metabolism.[16,17,27,28] As easily shown in the X-ray crystallographic structure of P450cam,[29] the prosthetic porphyrin moiety is protected from unfavorable reactions with oxidants by apoprotein as compared with the free synthetic porphyrins used in this study. Spectrophotometric monitoring has clearly shown that the porphyrins reacted with hydrogen peroxide either to form reactive oxygen species or to result in its oxidative deterioration.[25] The steric constraint on the porphyrin moiety could also explain less formation of phenol derivatives in the P450-catalyzed metabolism. The ester cleavage of parathion has been proposed to proceed *via* the reaction of a reactive phosphoxathiirane intermediate (Fig. 2) with water and the apoprotein makes the water molecule less accessible to the active site.[11] In contrast, although *O*,*O*,*O*-trimethyl phosphorothioate or phosphate could not be detected due to their trace amounts without radiolabeling, a methanol molecule was most likely to react with this intermediate, which would result in more formation of the phenol derivatives. In the reaction of **IV** with Fe(TPPCl$_8$$\beta$-Br$_8$)/H$_2O_2$, the slower transformation with less formation of the phenol than its oxon was observed, which might originate from less reaction caused by more bulkiness around the phosphorus of **IV** and that of Fe(TPPCl$_8$$\beta$-Br$_8$) imposed by many halogen atoms in the neighborhood of the central iron atom.

As demonstrated by the theoretical approaches on P450cam-catalyzed degradation of carbofuran,[5] the appropriate orientation of a substrate in the neighborhood of the active oxygen site is necessary for a site-specific oxidation. In the P450 system, not only the intrinsic reactivity but this spatial orientation is considered indispensable for an efficient oxidation. Rat metabolism studies of **I**–**IV**[16,17,27,28] have shown that the aryl methyl group of **I**, **III** and **IV** was stepwise oxidized but with no ring hydroxylation. The electron distribution of HOMO of each pesticide was found to be mainly localized at the P=S sulfur followed by the carbon atoms in the phenyl rings (16%, **I**; 40%, **II**; 54%, **III**; 9%, **IV**). The P450-catalyzed hydroxylation of an alkyl group was considered to proceed *via* hydrogen abstraction, but very little HOMO distribution at the aryl methyl groups of **I**, **III** and **IV** was estimated (<1.9%) by MNDO-PM3 calculations. In the case of **V**, the synthetic porphyrin predominantly oxidized the C–S sulfur as recently reported for aromatic and aliphatic sulfides,[30] followed by ester cleavage and desulfuration to form **Vd** and **Ve**. Although it was not clear which site, P=S or C–S sulfur, was more susceptible to oxidation, significant amounts of oxon and its sulfone in addition to **Va**, **Vb** and **Ve** and hydrolytic products were detected in rat[31] and rabbit[32] metabolism studies. These results indicated that the electronic character primarily controlled the porphyrin-mediated oxidation and the absence of steric constraint caused the different reac-

tion profiles from P450-catalyzed metabolism.

Since the f_E index could not elucidate the different reactivities of the pesticides, an alternative approach was undertaken. When pesticide and reactive porphyrin molecules react at the sulfur and oxygen atoms, respectively, the interaction energy at the initial stage, called the polarization energy, can be approximately described on the basis of the perturbation theory.[26] This energy consists of two terms, that is electrostatic and molecular orbital interactions between the reaction sites. The latter term is proportional to the electron distribution at the P=S sulfur and the active oxygen species in the porphyrin and inversely proportional to the difference of energy levels between the HOMO of a pesticide and the relevant MO of the porphyrin. Since the observed two pathways of pesticide were considered to proceed *via* the same intermediate as shown in Fig. 2, the following equation was proposed according to the perturbation theory to examine the oxidative reaction rates (k).

$$\log k = a + b \times f_E(S)/|E_L - c|$$

k is the first-order degradation rate of pesticide in hr^{-1}, E_L is the energy level of HOMO of pesticide in eV, $f_E(S)$ is the reaction index at the P=S sulfur atom, and a, b, and c are constants. Although the experimental data were not enough for regression, the following results were obtained for **I–IV** showing similar reaction profiles.

$Fe(TPPF_{20})$;
$\log k = -0.7316 - 0.0012 \times f_E(S)/|E_L - (-9.787)|$ (r=0.83)

$Fe(TPPCl_8)$;
$\log k = -2.063 - 0.273 \times f_E(S)/|E_L - (-9.257)|$ (r=0.99)

$Fe(TPPCl_8\beta\text{-}Br_8)$;
$\log k = -0.689 - 0.221 \times f_E(S)/|E_L - (-9.268)|$ (r=0.99)

Loew *et al.*[4] have reported the iterative extended Hückel calculations of the proposed oxygen-iron porphyrin complex in P450 and the two singly occupied MOs at about −10.3 eV are nearly degenerate and extremely delocalized at iron and oxygen atoms. The hamiltonian method of calculation is much different between the extended Hückel and MNDO-PM3 and hence, a direct comparison of computational results is impossible. However, it would be qualitatively possible to assume that these two singly occupied MOs participate in interactions with the HOMO of a pesticide in porphyrin-catalyzed oxidations. The energy level of the corresponding MO in the reactive porphyrin was estimated to be (−9.787 eV) for $Fe(TPPF_{20})$. Slightly higher values were obtained for $Fe(TPPCl_8)$ (−9.257 eV) and $Fe(TPPCl_8\beta\text{-}Br_8)$ (−9.268 eV), which might originate from the large structural differences at either *meso*- or β-positions of the porphyrin ring. The consistency of these values with that obtained for the P450 model should be further examined using the same computational methods. The obtained regression suggested that the electron distribution of the HOMO of a pesticide as well as its energy level controlled the porphyrin-mediated oxidation of organophsophorus pecticide.

Although our system could not fully demonstrate the metabolic oxidation catalyzed by P450, it was found to be very useful for estimating the primary oxidation reaction of an organophosphorus pesticide by the simple *in vitro* method.

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