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Multiple Active Intermediates in Oxidation Reaction Catalyzed by Synthetic Heme−Thiolate Complex Relevant to Cytochrome P450

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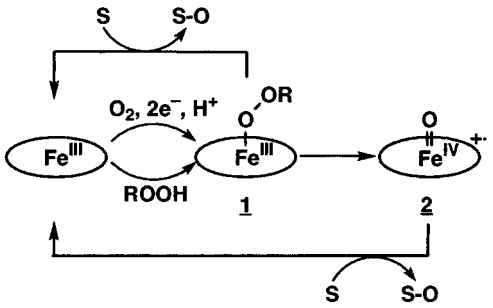
Abstract: We have studied oxidation reactions using a synthetic heme−thiolate (**SR** complex) in order to ascertain the contributions of multiple intermediates derived from heme−thiolate to the oxygen atom transfer reaction to substrate. First, degradation of peroxyphenylacetic acid (PPAA) was examined in the presence of various substrates. The O−O bond cleavage mode of PPAA was clearly dependent on the reactivity of the substrate, and an easily oxidizable substrate enhanced heterolytic O−O bond cleavage. Second, competitive oxidations of cyclooctane and cyclooctene were carried out with various peroxybenzoic acids containing a series of substituents at the para-position as an oxygen source. The ratios of alkane hydroxylation rate/alkene epoxidation rate were dependent on the nature of the para-substituent of the oxidant. We conclude that substrate and oxidant interact with each other during the oxygen atom transfer reaction, that is, oxidation reaction occurs before O−O bond cleavage, even in the reaction catalyzed by heme−thiolate, which is considered to promote O−O bond cleavage. The results of an ^{18}O-incorporation study that is frequently performed to determine the active intermediates derived from iron porphyrins were consistent with this conclusion.

Introduction

Cytochrome P450 is a group of heme-containing monooxygenases that play important roles in the metabolism of many physiological substrates and xenobiotics.[1] Because of their extremely strong oxidizing ability, much interest has been focused on P450 chemistry, and identification of the reactive intermediate responsible for oxygen atom transfer to substrate in catalytic oxidation by cytochrome P450 has been one of the major goals in bioinorganic chemistry. A high-valent oxo-iron porphyrin π-cation radical (called "compound I", **2** in Scheme 1), formally two-electron-oxidized from the ferric state, has been widely proposed as the active species.[1−3] Indeed, compound I is observable by spectroscopic techniques in the reactions of oxidant and many synthetic iron porphyrins ligated by Cl^-, AcO^-, MeOH, or other nonthiolate ligands[4] and in the reactions of enzymes such as peroxidase and catalase, although they also have a nonthiolate axial ligand.[5] Furthermore, Sligar et al. recently obtained the structure of an intermediate that would be consistent with an oxyferryl species resulting from X-ray irradiation of ferrous-dioxygen P450cam by the use of time-lapse X-ray crystallography.[6] However, the fact that a certain intermediate is generated by stoichiometric reaction in the absence of substrate does not necessarily imply that the

Scheme 1



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intermediate is responsible for the oxidation reaction even in catalytic reactions. The catalytic reaction in the presence of substrates could be quite different. In contrast to the conventional simple scheme, in which compound I is the sole reactive intermediate responsible for the oxidation reaction based on direct and indirect evidence,[7] recent studies suggest that the actual reaction mechanism is a more complex one, as shown in Scheme 1.[8,9] Machii et al. demonstrated that the active species responsible for the epoxidation is not always compound I in catalytic epoxidation by synthetic iron porphyrins and also showed that competitive epoxidation is a good indicator to discriminate the active species.[8a] Nam et al. studied the competitive epoxidation by synthetic iron porphyrins with hydroperoxides or peracids and demonstrated that two intermediates, **1** and **2** in Scheme 1, were both able to oxidize easily oxidizable substrates such as alkenes and that the nature of the reactive intermediates depended upon the rate of the O−O bond cleavage of **1**.[8b] Further, Nam et al. demonstrated that **1** could oxidize even nonactivated alkanes to give alcohol products.[9a] Collman et al. also provided evidence that the active intermediates in the oxidation reactions by iron porphyrins with iodosylbenzene derivatives as oxygen donors are iron porphyrin−oxidant complexes.[9b] In the case of P450 enzymes, site-specific mutagenesis studies performed by Vaz et al. and Newcomb et al. indicated that iron−hydroperoxide (**1**), iron−hydrogen peroxide (protonated **1**), and iron−oxo complex (**2**) all act as electrophilic oxidants in alkene epoxidation and alkane hydroxylation.[10]

However, these previous studies using synthetic hemes were carried out by use of nonthiolate synthetic hemes. Considering that axial thiolate coordination is a key feature of cytochrome P450 and that the axial ligand greatly affects both the reactivity of heme enzyme[11] and that of synthetic heme,[12] thiolate-ligated iron porphyrin would be clearly preferable as a model for reaction mechanism analysis relevant to P450. While Vaz et al. and Newcomb et al. obtained interesting results by using P450 enzymes and their mutants, synthetic model studies with thiolate-ligated iron porphyrin would be of value because one can chose various oxidants, substrates, and reaction conditions such as temperature, polarity of solvent, and so on, as required for analysis of each reaction.

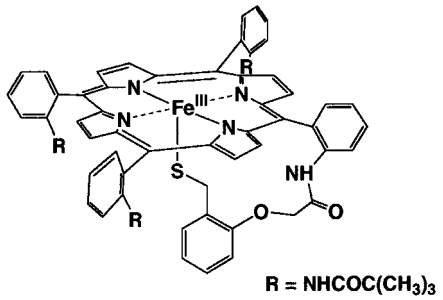

Figure 1. Structure of the SR Complex.

We synthesized the first synthetic heme−thiolate (**SR** complex,[12a] Figure 1) that retains thiolate coordination during catalytic oxidation and have found several remarkable thiolate axial ligand effects.[12a−c,13] In previous studies using SR, we measured the rates of substrate oxidation by SR with several alkyl hydroperoxides and found that the sixth thiolate ligand of **SR** induces a 50−250-fold increase in the oxidation rates compared with chloride ligand.[12a] We also examined the degradation of terminal oxidant mediated by **SR** and found that the thiolate ligand enhances the formation of the two-electron-oxidized intermediate in high yield without the assistance of acid or base.[13] To assess the effect of thiolate ligand on the oxidation reactivity, we examined the kinetic isotope effect and ^{18}O incorporation from ^{18}O-enriched oxidants in the *O*-demethylation reaction and examined the alkane oxidation rate/alkene oxidation rate ratio in the competitive oxidations of alkane and alkene catalyzed by **SR**, other synthetic iron-porphyrins, and cytochrome P450s. From these studies, we have established that the catalytic features of **SR** and P450s are very similar and that the thiolate ligand has a marked influence on the reactivity of the intermediate, although the electronic structure of the reactive intermediate derived from **SR** remains uncertain.[12b,c]

Now in the present study, we have carried out reaction analysis by use of **SR** with various oxidants and substrates in order to throw light on the nature of the reactive intermediate derived from **SR** that is truly responsible for the oxygen atom transfer reaction in catalytic oxidation.

Experimental Section

Materials. CH_2Cl_2 was distilled from CaH_2 before use. Peroxyphenylacetic acid and other substituted peroxybenzoic acids were prepared by a literature method[14] and purified by washing with 200 mM sodium phosphate buffer (pH = 6.5) and recrystallization from hexane. **SR** complex and Fe(TMP)Cl (5,10,15,20-tetramesitylporphyrinate iron(III) chloride) were prepared by a method reported previously.[12,15,16] Fe(TPFPP)Cl [5,10,15,20-tetrakis(pentafluorophenyl)porphyrinate iron(III) chloride] was purchased from Aldrich Chemical Co. and used without further purification. TBPH (2,4,6-tri-*tert*-butylphenol) was purchased from Tokyo Kasei and purified by silica gel column chromatography and recrystallization from EtOH−H_2O. Cyclooctane and cyclooctene were purchased from Tokyo Kasei and distilled from CaH_2 before use.

Instruments. GC/SIM (gas chromatography/selected ion monitoring) analyses were performed on a Hewlett-Packard 5890 Series II gas

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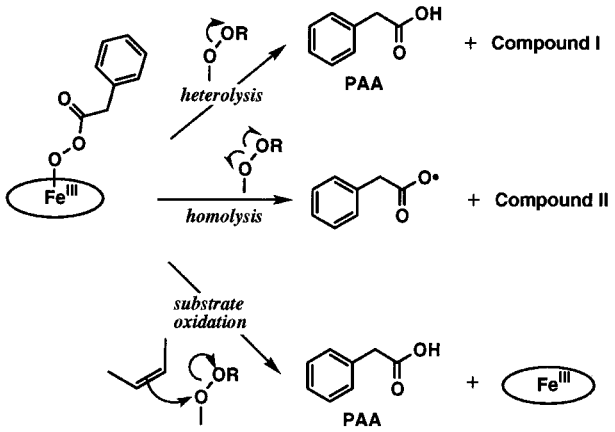
Scheme 2



chromatograph with a J&W Scientific DB-5 (30 m) capillary column and JEOL JMS-SX 102A mass spectrometer.

Analysis of the O−O Bond Cleavage Mode. A methylene chloride solution (0.8 mL) containing an iron porphyrin (0.125 mM) and a substrate (25 mM TBPH or cyclooctene or 250 mM cyclooctane) was prepared. The reaction was started by adding a methylene chloride solution of PPAA (0.2 mL, 0.5 mM) at 20 °C under Ar. The reaction was terminated by adding a methylene chloride solution of Ph_3P (0.05 mL, 4 mM) after 30 s (in the case that the iron porphyrin was **SR**) or 10 min [in the case that the iron porphyrin was Fe(TMP)Cl or Fe(TPPFP)Cl]. The reaction mixture was further stirred for 5 min, then a methylene chloride solution of diethylcatechol (0.2 mL, 0.1 mM) was added as an internal standard, and the products were analyzed by GC−SIM.

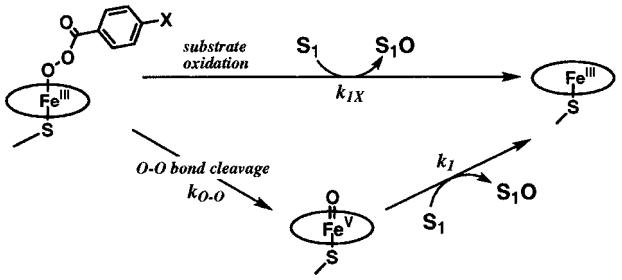
Competitive Oxidation. A methylene chloride solution (0.4 mL) containing **SR** complex (1.25 mM) and substrates (25 mM cyclooctene and 250 mM cyclooctane) was prepared. The competitive oxidation was started by adding a methylene chloride solution of a substituted peroxybenzoic acid (0.1 mL, 5 mM) at −15 °C under Ar. The reaction was terminated by adding a methylene chloride solution of Ph_3P (0.1 mL, 50 mM) after 1 min. The reaction mixture was further stirred for 5 min at 20 °C, then a methylene chloride solution of diethylcatechol (0.1 mL, 0.2 mM) was added as an internal standard, and the products were analyzed by GC−SIM.

Analysis of ^{18}O Incorporation from Bulk Water. CH_2Cl_2/CH_3OH (1:1, total volume 0.4 mL) solution or CH_2Cl_2/CH_3CN (1:1, total volume was 0.4 mL) solution containing iron porphyrin (0.625 mM) and cyclooctene (12.5 mM) and $H_2{}^{18}O$ (10%) was prepared. The reaction was started by adding a methylene chloride solution of mCPBA (0.1 mL, 10.0 mM) at various temperatures under Ar. The reaction was terminated by adding a methylene chloride solution of Ph_3P (0.05 mL, 25 mM) after 1 min (in the case that the iron porphyrin was **SR**) or 20 min [in the case that the iron porphyrin was Fe(TPPFP)Cl]. The reaction mixture was further stirred for 5 min, then a methylene chloride solution of diethylcatechol (0.1 mL, 0.2 mM) was added as an internal standard, and the products were analyzed by GC−SIM.

Results and Discussion

The present experiments were designed to throw light on the nature of the reactive intermediate. If the initially formed species (**1**, shown in Scheme 1) contributes to the oxidation reaction, the substrate and oxidant could interact with each other during the reaction. Specifically, substrates that can be readily oxidized by the active intermediate could apparently affect the O−O bond cleavage mode of oxidants, as shown in Scheme 2, and electron-donating or electron-withdrawing substituents of oxidants could affect the reactivity of the intermediate, such as substrate selectivity, reaction rate, and so on, as shown in Scheme 3. If

Scheme 3



a high-valent oxo−iron porphyrin complex is the sole intermediate responsible for oxygen transfer, such phenomena should not occur, i.e., the O−O bond cleavage mode of oxidants should be independent of the substrate, and the reactivity of the intermediate should be independent of the oxidant.

Effect of the Substrate on the Mode of O−O Bond Cleavage of PPAA. First, we investigated the mode of O−O bond cleavage mediated by **SR** in the presence of several substrates with a range of reactivity, i.e., TBPH, cyclooctene, and cyclooctane based on the hypothesis illustrated in Scheme 2. Their reactivity is in the order of TBPH > cyclooctene > cyclooctane. Many studies have been carried out on the mode of O−O bond cleavage of oxidants.[17] We chose to use peroxyphenylacetic acid (PPAA), which has frequently been used as a probe for this purpose,[18] because the cleavage mode can be easily examined by quantitative determination of degradation products derived from the oxidant. Heterolytic cleavage of the O−O bond affords phenylacetic acid, and homolytic cleavage affords toluene, benzyl alcohol, and benzaldehyde via the benzyl radical. The O−O bond of each acylperoxo intermediate cleaves with each inherent hetero/homo ratio, but direct reaction of acylperoxo intermediate and substrate affords phenylacetic acid and apparently affects the O−O bond cleavage mode. The reactions were performed in CH_2Cl_2, a relatively inert solvent that provides a hydrophobic environment around the complex, as is generally the case at the active sites of P450s. In each reaction, a large excess of the substrate and just 1 mol equiv of PPAA to **SR** were used, because it is necessary to minimize the decomposition of **SR** and to prevent secondary oxidation of the formed phenylacetic acid by the reactive intermediate. We confirmed the fact that secondary oxidation of the formed phenylacetic acid did not occur under this condition by another study (not shown). In the other porphyrin systems, the same amounts of oxidant and substrate as in the **SR** system were used to obtain comparable results.

In the oxidation reactions catalyzed by **SR** with PPAA, phenylacetic acid was predominantly formed (80−96% based on PPAA) as the degradation product derived from the oxidant in every reaction, compared with the reactions catalyzed by other synthetic iron porphyrins. This result means that the thiolate axial ligand enhances heterolytic O−O bond cleavage in all of the reactions with various substrates, as we previously reported.[13] However, significant differences in the yields of degradation

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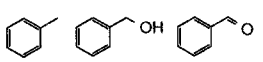
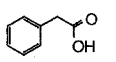
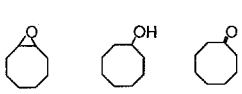
Table 1. Yield of Degradation Products Derived from Peroxyphenyacetic Acid Mediated by **SR**, Fe(TPP)Cl, and Fe(TPFPP)Cl in the Presence of Various Substrates[a]

		Homolysis[b]			Heterolysis[b]			Oxidation products		
Catalyst	Substrate					Hetero/homo ratio	Recovery[b]			
SR	TBPH	0.11	1.32	N. D.[c]	95.97	68.6	97.40			
	cyclooctene	0.21	1.32	3.04	91.67	19.9	96.24	22.27		
	cyclooctane	0.62	1.35	6.21	79.83	9.7	88.01		15.96	0.68
Fe(TMP)Cl	TBPH	0.14	11.20	0.66	81.04	6.8	93.04			
	cyclooctene	0.20	4.13	8.51	76.55	6.0	89.39	26.86		
	cyclooctane	0.39	4.47	14.98	72.97	3.7	92.81		3.95	1.64
Fe(TPFPP)Cl	TBPH	0.19	14.14	1.21	72.89	4.7	88.43			
	cyclooctene	0.25	5.73	13.67	67.11	3.4	86.76	55.01		
	cyclooctane	0.45	7.38	17.59	60.14	2.4	85.56		29.52	2.74

[a] Details of reaction conditions are described in the Experimental Section. All reactions were carried out at least in duplicate, and the data reported represent the average values. [b] Yield based on PPAA. [c] Not detected.

products derived from PPAA were observed, depending on the substrate, and the ratio of heterolysis to homolysis varied from 68.6 for TBPH to 9.7 for cyclooctane, as shown in Table 1. These results demonstrate that homolysis of the O−O bond of PPAA certainly occurs in the SR−peroxy acid system if the reactivity of the substrate is fairly low, even though heme−thiolate complex was considered to greatly enhance heterolytic O−O bond cleavage[13,19] and to afford the acid quantitatively, owing to the so-called "push effect".[20] The fact that the ratio of heterolysis to homolysis varies with substrate implies that there is an interaction between O−O bond cleavage and substrate oxidation, that is, the initially formed acylperoxo intermediate that corresponds to the hydroperoxo intermediate in P450 catalytic cycle contributes to the oxidation reaction, at least to some extent (although the degree of contribution of the acylperoxo intermediate is still uncertain), as shown in Scheme 2. We consider that electron transfer from the substrate to the acylperoxo intermediate derived from **SR** and PPAA, in association with the electron transfer from olefin to in situ generated compound I,[1,21] enhances heterolysis of the O−O bond. In the Fe(TMP)Cl or Fe(TPFPP)Cl systems, significant differences in the yields of degradation products derived from PPAA were also observed, depending on the substrate, and reflect the contribution of the respective acylperoxo intermediates to the oxidation reaction. The difference between these two systems is dependent on the electronic nature of the porphyrin ligand.

Effect of the Substituent of the Oxidant on the Reactivity of the Intermediate. Next, we carried out competitive oxidation of cyclooctane versus cyclooctene catalyzed by **SR** to examine the effect of an electron-donating or electron-withdrawing substituent of oxidants on the reactivity of the intermediate based on the hypothesis illustrated in Scheme 3. It has been generally accepted that alkane hydroxylation and alkene epoxidation involving the common active intermediate, compound I, proceed via different mechanisms. It has been proposed that the alkane hydroxylation proceeds by an H atom abstraction−hydroxyl radical recombination mechanism in the case of P450,[22] while alkene epoxidation proceeds via a one-electron-transfer mechanism in metalloporphyrin-catalyzed oxidation.[21] Therefore, it is expected that alkene/alkane competitive oxidation can be used as a probe for discrimination of differences in chemical properties among active intermediates, as Mansuy et al. suggested.[23] This competitive oxidation has been used in our

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Table 2. Competitive Oxidation of Cyclooctane versus Cyclooctene Catalyzed by **SR** with Various Peroxybenzoic Acids Containing a Series of Substituents at the Para-Position[a]

Oxidant X	Products yields [b] 1	2	3	Total yield [b]	Alkane oxidation / alkene oxidation (2 + 3) / 1
$-NO_2$	6.45	3.97	0.08	10.58	0.63
–CN	6.18	3.95	0.06	10.25	0.65
$-CF_3$	7.33	5.00	0.05	12.43	0.69
–Cl	6.93	5.35	0.07	12.42	0.78
–F	6.62	5.23	0.11	12.07	0.81
$-OCH_3$	8.25	6.34	0.08	14.75	0.78

[a] Details of reaction conditions are described in the Experimental Section. All reactions were carried out at least in duplicate, and the data reported represent the average values. [b] Yield based on oxidant.

previous studies in order to discriminate differences in chemical properties among active intermediates derived from various P450 enzymes or synthetic hemes that have various porphyrin ligands or axial ligands, including **SR** and **SR** derivatives, from the viewpoint that the high-valent oxo−iron porphyrin complex is the sole reactive intermediate.[12b,24]

In this report, we used this competitive oxidation in order to examine in detail the contribution of the acylperoxo intermediate to the oxidation reaction. We used six peroxybenzoic acids with a series of substituents at the para-position: *p*-nitroperoxybenzoic acid, *p*-cyanoperoxybenzoic acid, *p*-(tirfluoromethyl)-peroxybenzoic acid, *p*-chloroperoxybenzoic acid, *p*-fluoroperoxybenzoic acid, and *p*-methoxyperoxybenzoic acid. The reactions were performed in CH_2Cl_2 under argon and at −15 °C in order to prevent direct epoxidation of peroxybenzoic acid, although we confirmed that the direct epoxidation was negligible, because the oxygen transfer reaction catalyzed by **SR** occurs immediately after peroxybenzoic acid addition, compared with the gradual direct epoxidation (not shown). Just as in PPAA oxidation, a large excess of the substrate and just 1 mol equiv of oxidant to **SR** were used. Cyclooctanol, cyclooctanone, and cyclooctene oxide were determined as oxidation products. The allylic hydroxylation of cyclooctene was negligibly small in all cases.

As shown in Table 2, SR−peroxy acid systems effectively oxidized cyclooctane and yielded cyclooctanol and cyclooctanone in every reaction, despite the difficulty of C−H bond activation, as we previously reported,[12b] and the ratio values of oxidation products are similar to each other. However, Table 2 also shows significant differences in the ratio values (from 0.63 to 0.81) when different peroxybenzoic acids were used. These results indicate that the substituents of the oxidants affected the substrate selectivity of the reactive intermediate, that is, the oxidation reaction occurs to some extent before the O−O bond

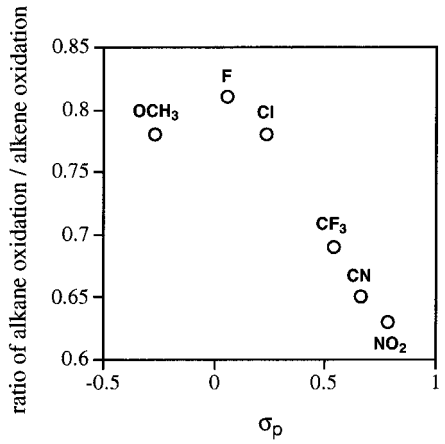

Figure 2. Hammett plot of the results of competitive oxidation of cyclooctane versus cyclooctene catalyzed by SR with various peroxybenzoic acids.

of the iron porphyrin−peroxybenzoic acid complex cleaves, because iron−oxo species should mediate the reactions with identical selectivity, regardless of its origin. This result is consistent with the result of PPAA oxidation, and now we can reasonably conclude that two distinct reactive intermediates contribute to substrate oxidation (at least, alkene oxidation) even in the reaction catalyzed by heme−thiolate, which has been considered to promote O−O bond cleavage.[25]

Quantitative evaluation of these substituent effects was performed by means of a Hammett plot, as shown in Figure 2. There seems to be a straightforward relationship between the ratio of alkane oxidation/alkene oxidation and σ_p of each substituent, except for the methoxy group. This significant correlation means that the change of the ratio is due to the electronic effect of the substituent of the peroxybenzoic acids used as terminal oxidants. If the acylperoxo intermediate of an iron porphyrin contributes to oxidation reactions, an electron-deficient oxidant would make the active intermediate more electron-deficient, so an electron-deficient oxidant such as *p*-nitroperoxybenzoic acid should enhances alkene epoxidation. In our oxidation system, in which **SR** was employed, electron-deficient oxidants certainly showed lower ratios of alkane oxidation/alkene oxidation compared with relatively electron-rich oxidants. Therefore, we concluded that the change in product ratio demonstrates that the acylperoxo intermediate contributes to oxygen atom transfer in oxidation reactions catalyzed by **SR**. Another possibility that affects the ratios of alkane oxidation/alkene oxidation is the contribution of compound II, formally one-electron-oxidized from the ferric state, formed by homolysis of the O−O bond. However, we could ruled out this possibility for the following reasons: If the oxidation reactions presented in this work proceeded via iron−oxo intermediates and if compound II formed by homolysis contributed to these oxidation reactions, an electron-deficient oxidant such as nitroperoxybenzoic acid should exhibit a higher ratio of alkane/alkene oxidation compared with a relatively electron-rich oxidant. This is because compound II dose not mediate alkane hydroxylation, so an electron-deficient oxidant that enhances heterolysis of the O−O bond should enhance generation of compound I and should enhance alkane hydroxy-

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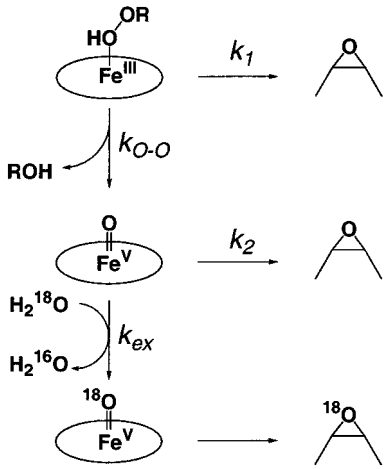
Scheme 4



lation. However, we obtained the opposite result in our work. If this change of ratio was caused by the different reactivity of each acylperoxo intermediate that was formed by each terminal oxidant, we can readily rationalize our results as described above.

^{18}O Incorporation Study from Bulk Water to Oxide Product in the Epoxidation Reaction Catalyzed by SR. Since isotopically labeled water, $H_2{}^{18}O$, has been frequently used as a probe for the formation of iron−oxo intermediates in oxidation reactions catalyzed by iron porphyrins, as shown in Scheme 4,[26] we examined the **SR**-catalyzed epoxidation of cyclooctene with mCPBA in the presence of $H_2{}^{18}O$ to determine whether an iron−oxo intermediate is involved in the oxidation reactions by analyzing the extent of ^{18}O incorporation from labeled water into the product. If the iron−oxo intermediate is generated in the reaction and if the rate of oxygen exchange between the iron−oxo intermediate and bulk water is comparable to the rate of oxygen transfer from the iron−oxo intermediate to substrate, the oxide product should contain the labeled oxygen.

First we carried out this study in methanol-containing solvent, as this is frequently used for ^{18}O incorporation studies. Under this condition, ^{18}O was incorporated into the oxide product generated by Fe(TPFPP)Cl-catalyzed epoxidation of cyclooctene with mCPBA in the presence of $H_2{}^{18}O$, depending on the reaction temperature (shown in Table 3), as Nam et al. previously reported.[27] However, the epoxide product was not formed in the reaction catalyzed by **SR** under this condition. This is because active intermediates derived from **SR** have extremely strong oxidizing ability and readily oxidize methanol. Therefore, we carried out this study in acetonitrile-containing solvent. Under this condition, ^{18}O incorporation into the epoxide product in the reaction catalyzed by Fe(TPFPP)Cl also occurred, as in the reaction in methanol-containing solvent, although the ^{18}O enrichment was smaller. In contrast, as shown in Table 3, ^{18}O was *not* incorporated in excess of the natural abundance into the oxide product formed by **SR**-catalyzed epoxidation of cyclooctene with mCPBA in the presence of $H_2{}^{18}O$, suggesting that the oxygen of the active intermediate derived from **SR** and mCPBA did not exchange with bulk water. In this study, incorporation of labeled oxygen can be regarded as indirect evidence of the formation of iron−oxo intermediates. However, the lack of oxygen exchange does not necessarily rule out the formation of iron−oxo intermediates. In this study, there might be several reasons for the lack of oxygen exchange. As shown in Scheme 4, lack of oxygen exchange means $k_1 \gg k_{O-O}$ (an iron−oxo intermediate is not formed) or $k_2 \gg k_{ex}$ (although an iron−oxo intermediate is formed, the rate of oxygen exchange is much smaller than the rate of oxygen transfer from the iron−oxo intermediate to the substrate). In addition, Nam et al. reported that when the axial position to the oxo group of the iron−oxo intermediate is occupied by a ligand such as imidazole, oxygen exchange of the iron−oxo intermediate with bulk water is greatly inhibited, because the six-coordinated iron−oxo intermediate does not have an appropriate binding site for water.[27] Therefore, the above results alone are not a critical test of our hypothesis described in Scheme 1, but they are consistent with the conclusion based on the other studies described here.

Table 3. Percentage of ^{18}O Incorporation from Bulk Water into the Oxide Product in the Epoxidation of Cyclooctene Catalyzed by **SR** and Fe(TPFPP)Cl with MCPBA[a]

catalyst	solvent	temp (°C)	% of ^{18}O in oxide product	% yield of oxide product[b]
SR	CH_2Cl_2/CH_3OH	25	−	ND[c]
		0	−	ND[c]
		−40	−	ND[c]
	CH_2Cl_2/CH_3CN	25	ND[d]	4.6
		0	ND[d]	5.8
		−40	ND[d]	6.4
Fe(TPFPP)Cl	CH_2Cl_2/CH_3OH	25	32.0	56.8
		0	12.1	66.0
		−40	4.6	86.4
	CH_2Cl_2/CH_3CN	25	5.0	74.3
		0	2.7	88.5
		−40	0.8	91.0

[a] Details of reaction conditions are described in the Experimental Section. All reactions were carried out at least in duplicate, and the data reported represent the average values. [b] Based on mCPBA. [c] No product peak was detected by GC−SIM. [d] ^{18}O incorporation in excess of the natural abundance was not detected.

Conclusion

Our results clearly demonstrate that the initially formed acylperoxo−heme−thiolate complex contributes to oxygen atom transfer in oxidation reactions catalyzed by **SR** in a hydrophobic environment resembling the hydrophobic active sites of P450s, although the respective contributions of the acylperoxo complex and pure iron−oxo complex to the oxidation reaction are still uncertain. In our reaction system, each terminal oxidant forms an intermediate, affording multiple **SR**−acylperoxo complexes that have distinct oxidizing abilities. This result supports the recent proposal about the reaction mechanism of cytochrome P450, i.e., that iron−hydroperoxide, iron−hydrogen peroxide, and iron−oxo complex act as multiple electrophilic oxidants in alkene epoxidation and alkane hydroxylation.[10] Because the molecular structure, hydrophobic environment of the reaction site, and oxidation reactivity of **SR** and P450 are very similar, we suggest that the oxidation reactions in the catalytic cycle of P450 probably occur in the same manner.

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Supporting Information Available: The time course of the oxidation reaction catalyzed by SR and Fe(TPFPP)Cl at low temperature by which we determined the reaction time for each complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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