Heterolytic O–O Bond Cleavage of Peroxy Acid and Effective Alkane Hydroxylation in Hydrophobic Solvent Mediated by an Iron Porphyrin Coordinated by Thiolate Anion as a Model for Cytochrome P-450

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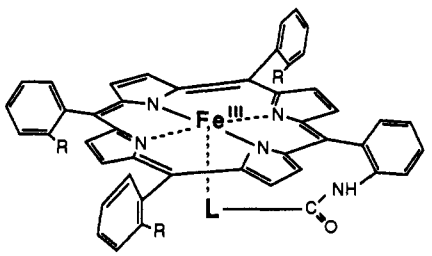
Steroid biosynthesis and metabolism of aliphatic xenobiotics in biological systems involve alkane hydroxylation processes which are mediated by the ubiquitous cytochrome P-450.[1] Among many heme enzymes, only P-450 can hydroxylate unactivated alkanes, although heme is a common moiety mainly responsible for these enzyme activities. The distinctive structural features of P-450 are the unusual thiolate coordination to heme and also the extreme hydrophobicity of its active site.[1] Our interest has been focused on the relative effect of an axial thiolate ligand on the reactivity of heme in lipophilic media as a model of the P-450 pocket. The iron porphyrin ligated by an alkyl thiolate anion (**SR** complex) previously reported by us is a unique one which retains its axial thiolate coordination during catalytic oxidation reactions.[2,3] The question of whether P-450 cleaves the O–O bond of heme–peroxo complex heterolytically or homolytically is one of the most significant points of current interest in P-450 chemistry.[1,4] We report here that **SR** complex cleaves the O–O bond of peroxy acids heterolytically even in highly hydrophobic solvents and the formed active intermediate can efficiently hydroxylate aliphatic hydrocarbons.



SR : L= -S$^-$-CH_2-(*o*-phenylene)-OCH_2-

1 : L= -N⟨ ⟩N-$(CH_2)_4$-, (CH_3COO^-)

R = -NHC(=O)C$(CH_3)_3$

A new iron porphyrin axially coordinated by imidazole intramolecularly (**1**) was prepared in order to compare the effect of imidazole as an axial ligand with that of thiolate. The structure of complex **1** is a modification of the intramolecularly imidazole-ligated heme prepared by Collman and co-workers,[5] and **1** was synthesized according to almost the same procedure used for **SR**.[6] The modes of O–O bond cleavage mediated by iron porphyrins were examined by using peroxyphenylacetic acid (PPAA), which has frequently been used as a probe for this purpose.[4,6] The oxidation of 2,4,6-tri-*tert*-butylphenol (TBPH) by PPAA catalyzed by hemes affords phenylacetic acid (PAA) when the O–O bond of PPAA breaks heterolytically (eq 1) or

$$Fe^{III}(Por) + PhCH_2CO_3H \xrightarrow{heterolysis} O{=}Fe^{IV}(Por)^{\bullet+} + PhCH_2CO_2H \quad (1a)$$

$$O{=}Fe^{IV}(Por)^{\bullet+} + 2TBPH \rightarrow Fe^{III}(Por) + 2TBP^{\bullet} + H_2O \quad (1b)$$

affords toluene and benzyl alcohol *via* benzyl radical in the case of homolysis (eqs 2a–c). It is less likely that a part of PAA is

$$Fe^{III}(Por) + PhCH_2CO_3H \xrightarrow{homolysis} HO{-}Fe^{IV}(Por) + PhCH_2COO^{\bullet} \quad (2a)$$

$$PhCH_2COO^{\bullet} \xrightarrow{very\ fast} PhCH_2^{\bullet} + CO_2 \quad (2b)$$

$$PhCH_2^{\bullet} \xrightarrow[X \quad Y]{} PhCH_3\ (X = TBPH,\ Y = TBP^{\bullet})$$
$$\text{or } PhCH_2OH\ (X = HO{-}Fe^{IV}(Por),\ Y = Fe^{III}(Por)) \quad (2c)$$

$$HO{-}Fe^{IV}(Por) + TBPH \rightarrow Fe^{III}(Por) + TBP^{\bullet} + H_2O \quad (2d)$$

formed by the reaction of $PhCH_2COO^{\bullet}$ with TBPH since $CH_3COO^{\bullet}$, which is much more stable than $PhCH_2COO^{\bullet}$, is known to decarboxylate very fast ($k > 10^9$/mol) even in the presence of phenols.[7,8] The presence of TBPH prevents the further oxidation of PAA or PPAA by the formed reactive intermediate (eqs 1b and 2d). Therefore, PAA formation should be caused exclusively by heterolytic cleavage of PPAA, and toluene and benzyl alcohol formation should be due to homolysis. The reaction profiles of this catalytic reaction were compared among three iron porphyrins *in benzene*, of which the hydrophobicity is presumed to be close to that of the environment of P-450's active site[1] (Table I). **SR** gave PAA quantitatively (run 1), while iron(III) tetraphenylporphyrin chloride (Fe(TPP)Cl) mainly catalyzed the formation of toluene, benzyl alcohol, and carbon dioxide (run 2). Complex **1** showed moderate reactivity, intermediate between **SR** and Fe(TPP)Cl (run 3). The peroxy acid very slightly decomposed **SR** complex (<2%) in this reaction to afford a high-spin iron porphyrin (named dec-**SR**), probably by oxidation of the thiolate ligand, and 98% of **SR** remained. The isolated dec-**SR** was not so efficient a catalyst for cleavage of the O–O bond of PPAA as **SR** (k_{cat}(dec-**SR**)/k_{cat}(**SR**) = 0.13) and mediated the formation of a considerable amount of toluene (run 4). Thus, dec-**SR** does not seem to be involved in the exclusive PAA formation by **SR**. The prior addition of PAA (1 equiv to PPAA) greatly enhanced heterolysis of PPAA by Fe(TPP)Cl (run 6), though it did not affect the product profile and the reaction rate in the case of **SR** (run 5). The use of peroxylauric acid instead of PPAA resulted in an analogous reaction pattern (runs 7, 8). Therefore, we can unambiguously conclude from these data that **SR** breaks the O–O bond of peroxyacids *heterolytically in benzene*. This result indicates that the thiolate ligand enhances heterolytic cleavage of peroxy acid–iron porphyrin complex even in highly hydrophobic media[10] without the assistance of acid or

(1) For recent reviews: (a) Ortiz de Montellano, P., Ed. *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Plenum Press: New York, 1986. (b) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255. (c) Dawson, J. H. *Science* **1988**, *240*, 433.

(2) Higuchi, T.; Uzu, S.; Hirobe, M. *J. Am. Chem. Soc.* **1990**, *112*, 7051.

(3) We named the iron porphyrin complex "swan-resting" form porphyrin because the shape topologically suggested a swan resting, burying its head in its feathers. The name of the complex is thus abbreviated as **SR**.

(4) White, R. E.; Sligar, S. G.; Coon, H. J. *J. Biol. Chem.* **1980**, *255*, 11108.

(5) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Bunnenberg, E.; Linder, R. E.; LaMar, G. N.; Gaudio, J. D.; Lang, G.; Spartalian, K. *J. Am. Chem. Soc.* **1980**, *102*, 4182.

(6) (a) Traylor, T. G.; Lee, W. A.; Stynes, D. V. *J. Am. Chem. Soc.* **1984**, *106*, 755. (b) Traylor, T. G.; Tsuchiya, S.; Byun, Y-S.; Kim, C. *J. Am. Chem. Soc.* **1993**, *115*, 2775 and references cited therein.

(7) Braun, W.; Rajbenbach, L.; Eirich, F. R. *J. Phys. Chem.* **1962**, *66*, 1591.

(8) The phenylacetoxy radical is known to decompose extremely quickly relative to the rate of diffusion and many have no effective individual existence.[9]

(9) Traylor, T. G.; Xu, F. *J. Am. Chem. Soc.* **1990**, *112*, 178.

(10) The use of chlorobenzene as the solvent in lieu of benzene resulted in almost the same reaction pattern (data not shown).

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Table I. TBPH Oxidation with Peroxy Acid Catalyzed by Iron Porphyrins

run	iron porphyrin	peroxy acid	products (yield, %)[a]			
1	**SR**	PPAA	PAA (98 ± 2)	$PhCH_3$ (0)	$PhCH_2OH$ (0)	TBP•(k_{cat} = 12.5)[b]
2	Fe(TPP)Cl	PPAA	PAA (9 ± 2)	$PhCH_3$ (61)	$PhCH_2OH$ (24 ± 4)	TBP•(k_{cat} = 0.043)[b]
3	**1**	PPAA	PAA (57 ± 2)	$PhCH_3$ (15)	$PhCH_2OH$ (0)	TBP• (k_{cat} = 0.33)[b]
4	dec-**SR**	PPAA	PAA (70)	$PhCH_3$ (22)	$PhCH_2OH$ (0)	TBP• (k_{cat} = 1.67)[b]
5	**SR**	PPAA + PAA[c]	PAA (98 ± 1)[d]	$PhCH_3$ (0)	$PhCH_2OH$ (0)	TBP• (k_{cat} = 12.1)[b]
6	Fe(TPP)Cl	PPAA + PAA[c]	PAA (83 ± 12)[d]	$PhCH_3$ (1)	$PhCH_2OH$ (14)	TBP• (k_{cat} = 0.083)[b]
7	**SR**	peroxylauric acid		lauric acid (89)		
8	Fe(TPP)Cl	peroxylauric acid		lauric acid (25)		

[a] These results were carried out in benzene in 25 °C under argon for 10 min. PPAA, $PhCH_2CO_3H$; PAA, $PhCH_2CO_2H$. [Fe(Por)] = 0.10 mM; [peracid] = 1.0 mM; [TBPH] = 10 mM. Benzaldehyde was not detected. Otherwise as noted. Yields are based on the oxidants used. Each value represents mean ± SD of two determinations except for runs 4, 7, and 8 (one determination). Products were determined by GLC and/or GCMS.[b] k_{cat} = the initial rate of TBP• formation (turnover numbers/s). [c] One equivalent amount of PAA to PPAA was added. [d] Yield based on two equivalent amounts to the added PPAA.

Table II. Hydroxylation of Alkanes with Peroxy Acid Catalyzed by Iron Porphyrins

run	iron porphyrin	substrate	peroxy acid	products (yield, %)[a]		
1	**SR**	adamantane	mCPBA	1-adamantanol (80 ± 1)	2-adamantanol (8)	
2	Fe(TPP)Cl	adamantane	mCPBA	1-adamantanol (9 ± 2)	2-adamantanol (2)	
3	**1**	adamantane	mCPBA	1-adamantanol (34 ± 4)	2-adamantanol (5)	
4	**SR**	adamantane	PPAA	1-adamantanol (61 ± 4)	2-adamantanol (7)	PhCHO + $PhCH_2OH$ (2 ± 1)
5	Fe(TPP)Cl	adamantane	PPAA	1-adamantanol (11 ± 4)	2-adamantanol (2)	PhCHO + $PhCH_2OH$ (13 ± 3)
6	**SR**	cyclohexane	mCPBA		cyclohexanol (30)	
7	Fe(TPP)Cl	cyclohexane	mCPBA		cyclohexanol (3)	
8	**1**	cyclohexane	mCPBA		cyclohexanol (16)	

[a] These reactions were carried out in benzene at 25 °C under argon for 10 min. PPAA, $PhCH_2CO_3H$; mCPBA; *m*-chloroperbenzoic acid. [Fe(Por)] = [peracid] = 1.0 mM; [adamantane] = 0.50 M; [cyclohexane] = 3.0 M. Yields are based on the oxidants used. Each value represents mean ± SD of two determinations except for runs 6, 7, and 8 (one determination). Products were determined by GLC and/or GCMS. Ketone form product was not detected in any case.

base. In contrast, it was deduced from our result that Fe(TPP)Cl catalyzes the homolysis of peroxy acid in benzene. The conclusion concerning the O–O bond homolysis by Fe(TPP)Cl in benzene is consistent with the results described by Groves, Watanabe, and co-workers.[11] There has been a report by White *et al.* proposing a mechanism involving homolysis of the O–O bond for P-450, based on the result of experiments using the enzyme itself and PPAA as a peroxide probe.[4] They observed benzyl alcohol formation in the oxidation of aliphatic substrates with PPAA catalyzed by P-450s. However, they did not confirm that P-450 itself is really responsible for the benzyl alcohol formation, rather than P-420, which is produced by the reaction of PPAA with P-450.[12]

It is expected that a more strongly electron-donating axial ligand would more greatly enhance both heterolytic O–O bond scission and its rate. Therefore, the order of donative character of the examined ligands can be estimated to be as follows: thiolate > imidazole ≫ chloride anion.

Next, the reactivity of the active species formed by cleavage of the O–O bond of peroxy acid–iron porphyrin complexes was examined by using unactivated alkanes as substrates in benzene (Table II). The amount of each peroxy acid used was 1 mol equiv to the iron porphyrins because it is necessary to minimize the ratio of decomposition of **SR** complex and the peroxy acid and the secondary oxidation of the formed acid by active species. The **SR** complex catalyzed the hydroxylation of adamantane so efficiently that the yield of adamantanols based on the used mCPBA reached 88% (run 1). In the reaction, 80% of **SR** in the reaction mixture was confirmed to remain undecomposed by EPR and UV–vis spectroscopies. On the other hand, only a low or moderate yield of adamantanol was obtained by catalysis with Fe(TPP)Cl or complex **1**, although mCPBA was completely consumed (runs 2, 3). Both Fe(TPP)Cl and complex **1** also retained their structures after completion of the reactions. Almost no bis(*m*-chlorobenzoyl) peroxide was detected in any reaction mixture. A similar tendency was also observed when PPAA was used as an oxidant (runs 4, 5) or when cyclohexane was used as a substrate (runs 6–8). In CH_2Cl_2, in which medium Fe(TPP)Cl can cleave the O–O bond of PPAA heterolytically,[11b] a great similarity of catalytic reactivity between **SR**, **1**, and Fe(TPP)Cl was observed in terms of the hydroxylation of adamantane by mCPBA (the ratio of the yield of adamantanols = 2:2:1). The degree of activity of iron porphyrins for heterolysis of PPAA in benzene is thought to correlate positively with the degree of hydroxylation activity toward alkanes. It is therefore highly probable from these experiments that a two-electron-oxidized species ($O{=}Fe^{IV}(por)^{\bullet+}$ or $O{=}Fe^{V}(por)$) is formed in high yield by the heterolytic O–O bond scission of peroxy acid–**SR** complex and that the intermediate has very high activity to hydroxylate aliphatic hydrocarbons. Perhaps acyloxy radical and HO–$Fe^{IV}(por)$ intermediate, which are formed by the homolysis of Fe(TPP)Cl–peroxy acid complex in benzene, have insufficient reactivity toward alkanes.[13] The large enhancement of the reactivity of iron porphyrin in alkane hydroxylation in hydrophobic media by RS^- coordination provides a rational explanation for the role of thiolate coordination of heme in cytochrome P-450.

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Supplementary Material Available: Experimental procedures and spectral data for compound **1** and dec-**SR** (1 page). Ordering information is given on any current masthead page.

(11) (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.

(12) Our preliminary observations of UV–vis spectra of heme (Fe^{2+})–CO complexes have revealed that most of P-450 is converted into P-450 during P-450 reactions using PPAA as an oxidant under almost the same conditions as those described in ref 4. It is therefore suspected that benzyl alcohol is formed by the reaction of PPAA with P-420, of which the heme has no axial thiolate ligand.

(13) Almost no ^{18}O was incorporated into the formed 1-adamantanol in the hydroxylation of adamantane by PPAA catalyzed by **SR** on Fe(TPP)Cl in benzene saturated with $H_2{}^{18}O$. The results do not suggest that the type of active species *which conducts adamantane hydroxylation* in the reaction catalyzed by Fe(TPP)Cl differs from that in the case of **SR**.