New Insight into Reaction of Iron(III)-peroxide Adduct with Alkanes: an Alternative Model for Cytochrome P-450 and Methane Monooxygenase

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Iron(III)-peroxide Adduct, Alkane Functionalization, Activation of Peroxide Adduct, Electrophilicity of Metal-peroxide

A new mechanism for alkane functionalization by iron(III)-peroxide adducts was proposed, and the importance of electrophilic nature of the metal-peroxide adduct with η^1-coordination mode was emphasized. This idea suggests that formation of high-valent iron-oxo species occurs most likely when the metal-peroxide intermediate is activated through electronic interaction with both the peripheral organic group and substrate; the latter two act as an electron donor to the peroxide adduct.

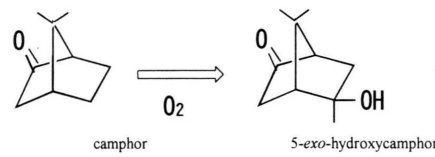
One of the remaining frontiers in organic chemistry is the direct functionalization of saturated hydrocarbons. Considerable progress has been made in understanding the chemical requirements for transition metal "activation" of carbon-hydrogen bonds (Hill, 1989). The catalytic cycle that oxidizes a hydrocarbon RH to an alcohol ROH employing P-450 is also a well-established reaction (Montellano, 1986; Sono *et al.,* 1996).

Cytochrome P-450s constitute a large group of enzymes and contain a conserved cysteine, the thiolate group of which is ligated to the haem iron (Poulas *et al.,* 1987). Cytochrome P-450$_{CAM}$ (P-450$_{CAM}$, CYP101) is a monooxygenase that catalyzes the hydroxylation of camphor in the bacterium *Pseudomonas putida* (see Table I). It was the first member of a ubiquitous family of P-450 isoenzymes for which a crystal structure was reported. Extensive experimental investigation has helped to elucidate the steps in the P-450 enzymatic cycle thought to be common to all isozymes. Fig. 1 indicates (Groves and Nemo, 1983; Gunter and Turner, 1991) these steps schematically and shows cytochrome P-450's hydroxylate substrates *via* an enzymatic cycle which involves (i) entry of the substrate, (ii) displacement of most or all of the

Table I. Oxygen and NADH consumption rates of wild-type and mutant cytochrome P-450$_{CAM}$ in a reconstituted system and the amount of products formed (Imai, *et al.* 1989).

Mutant site-residue	Rate of consumption Oxygen (μmol/min x μmol heme)	NADH	Product formed per O_2 consumed 5-OH-CAM[a] in %	H_2O_2
252-Thr(wild)	1350	1380	97	3
252-Ala	1150	1180	5	89
252-Gly	1090	1090	3	88
252-Ser	830	830	85	15
252-Val	260	250	24	51

[a] 5-OH-CAM; 5-*exo*-hydroxycamphor.



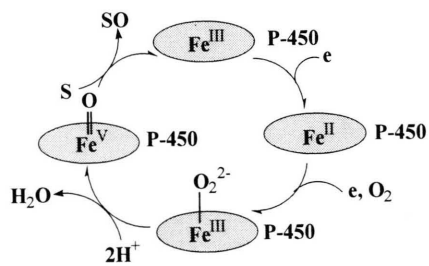


Fig. 1. Mechanism of oxygen activation in cytochrome P-450 by Groves and Nemo (1983).

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substrate cavity water, (iii) a change of ferric heme spin state upon displacement of the sixth ferric heme water, (iv) a change in the redox potential toward one-electron reduction, *via* a complex electron transport system, (v) a one-electron reduction to a ferrous heme followed by (vi) entry and binding of molecular oxygen to the ferrous state, (vii) a second one-electron reduction, (viii) formation of the putative reactive intermediate, high-valent iron-oxo species, and (ix) hydrogen radical abstraction by the ferryl oxygen from the substrate followed by radical combination to produce the hydroxylated products and regeneration of the resting state of the enzyme. In addition to the nature of the reactive intermediate itself, its mechanism of formation from the ferrous dioxygen species is also still under active investigation. Among the major unresolved questions are the (1) the role and identity of the binding residues, if any, involved in the formation of the reactive intermediate and (2) the concertedness of the second electronic reduction and O–O bond cleavage to form the ferryl oxygen species. One common hypothesis is that hydrogen bonding and/or proton donation stabilizes the initial ferrous dioxygen complex and facilitates the O–O bond cleavage in the twice reduced state. However, a high-valent iron(V)-oxo species in Fig. 1 has not been yet identified or characterized for the cytochrome P-450's (Harris and Loew, 1994).

Sequence alignments of P-450's indicate that the threonine residue is a highly conserved residue at sequence location 252. In P-450_{CAM}, this threonine forms part of the dioxygen binding groove in the substrate binding site. Imai *et al.* have reported that the efficiency in conversion of camphor to 5-exo-hydroxycamphor drops to only 5–6% and electrons are channeled to produce hydrogen peroxide and water, when the highly conserved active site Threonine-252 is replaced with alanine or glycine (see Table I) (Imai *et al.*, 1989). The cyrstal structure of a P-450_{CAM} site-directed mutant in which the active site Thr252 has been replaced with an alanine(Thr252Ala) has been determined by Raag *et al.* (1991). This has revealed that a solvent molecule not present in the native enzyme is positioned in the dioxygen-binding region of the mutant enzyme active site, and thus solvent protons appear to be much more accessible to dioxygen in the mutant than in the wild-type enzyme, factor which may promote hydrogen peroxide and/or water production instead of substrate hydroxylation. These facts suggest that promoted O–O bond cleavage by hydrogen bonding and/or proton donation in the twice reduced state (see Fig. 1) is unlikely. Above consideration may be supported by the fact that a mutant enzyme with a methoxy group in place of the hydroxy group of threonine-252(OMe-mutant) retains a considerably high monooxygenase activity, yielding a stoichiometric amount of the product to that of the oxygen consumed (Kimata *et al.*, 1995).

Harris *et al.* (1994) have performed the molecular dynamics simulations on the ferrous bound form of wild type P-450_{CAM}, and the results indicate a time-dependent bimodal interaction of Thr252 with both Gly248 and the terminal oxygen of the bound dioxygen. The hydrogen bonding interaction of Thr252 with these two moieties is "anticorrelated" in the sense that the breaking of the Thr252–Gly248 hydrogen bond is concurrent with formation of the Thr252-dioxygen interaction. Because it seems quite unlikely that proton is related with the cleavage of O–O bond as described above, the bonding of Thr252-dioxygen should have an another important meaning in dioxygen activation in P-450. In order to investigate the function of Thr252 and its O-methyl derivative in the oxygenation reaction, we have compared the reactivities towards alkane functionalization of the iron(III)-peroxide adducts where the peroxide ion can interact with the organic group nearby; examples are illustrated in Fig. 2 (Nishida *et al.*, 1995; Ito *et al.*, 1996a). The figure in the left side of Fig. 2 is our model for P-450_{CAM}, which may be represented by the complexes with (etapy) and also with (bbimae), where in the latter complex the coordination of alcohol group to an iron(III) is confirmed (Takahashi *et al.*, 1985). The peroxide adduct shown in the right side is indicating our model for the OMe-mutant.

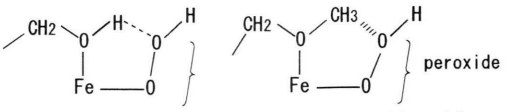


Fig. 2. Interaction between Fe(III)-peroxide adduct and peripheral group.

Materials and Methods

Materials

We have prepared linear (μ-oxo)diiron(III) compounds with the ligands shown in Fig. 3 (Nishida *et al.,* 1995); the compound with (etapy) was obtained in this study. Crystal structure determinations of several compounds have revealed that the structural features of the compounds are essentially the same as those reported for the (tpa)-complex (Leising *et al.,* 1993; Kojima *et al.,* 1993) with linear μ-oxo bridge; as an example the ORTEP drawing of the (epy)-complex is illustrated in Fig. 4 (Nishida *et al.,* 1995).

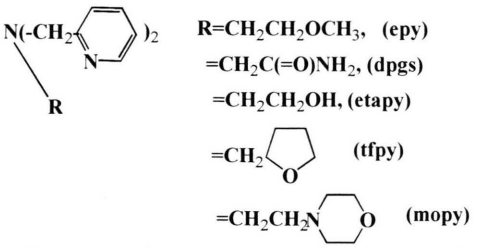


Fig. 3. Chemical structures of the ligands cited in this paper and their abbreviations.

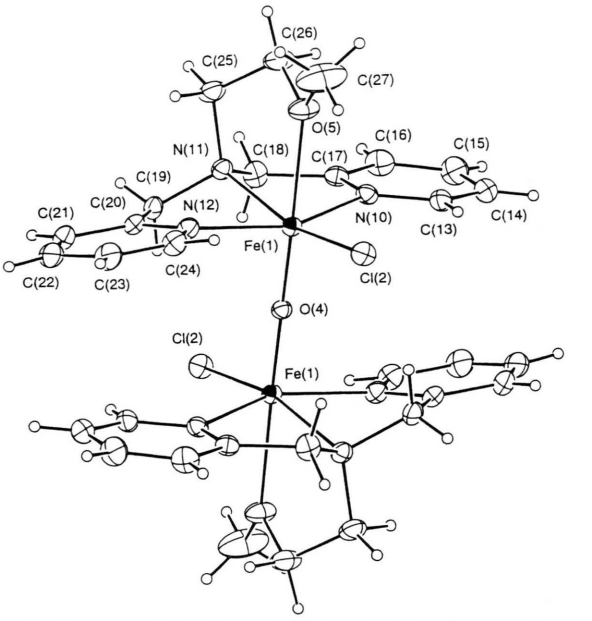


Fig. 4. ORTEP drawing of $Fe_2O(epy)_2Cl_2^{2+}$ complex. Selected bond distances are: Fe(1)–O(4), 1.777(1); Fe(1)–Cl(2), 2.287(1); Fe(1)–O(5), 2.264(2); Fe(1)–N(10), 2.140(2); Fe(1)–N(11), 2.205(2); Fe(1)–N(12), 2141(2) Å.

Catalase-like function of complexes

All reactions were performed at 20 °C in a 10 cm^3 reactor containing a stirring bar under air (Ito *et al.,* 1996a). The flask containing an iron(III) complex (10 μmol) solution (5 ml, acetonitrile) was closed with a rubber septum. Hydrogen peroxide solution (1 ml, commercial 30% aqueous solution was diluted to 1 M solution by acetonitrile) was injected through the septum with a syringe. The reactor was connected to a graduated burette filled with water and dioxygen evolved was measured at appropriate time by volumetry. The theoretical quantity of dioxygen molecule, which should be evolved under our experimental conditions, is *ca.* 16 ml, which was exemplified by the authentic experiment by the use of MnO_2 as a catalyst.

Oxygenation reaction of cyclohexane in the presence of iron(III) complex and hydrogen peroxide

In a typical run, an acetonitrile solution (20 ml) containing iron(III) complex with (etapy) and/or (bbimae) (0.05 mmol) and cyclohexane (840 mg) was added to an acetonitrile solution (10 ml) containing hydrogen peroxide (1.13 g of commercial 30% aqueous solution), and was kept to stand for at room temperature, and the oxygenated products were determined by GC. Cyclopentanone was used as an internal standard (Ito *et al.,* 1996a).

Molecular orbital calculations

The MNDO/AM1 calculations were performed for methylethylether, and the positional parameters of the compound was optimized by the use of the AM1 program (Ito *et al.,* 1996a). MO calculations for $Fe(NH_3)_4$(dimethylether)(HO_2^-) and $Fe(NH_3)_4$(methanol)(HO_2^-) were performed by the use of EHMO method reported by Hoffmann *et al.* (1977), where the parameters used for the iron(III) ion are the same as those used in the literature.

Results and Discussion

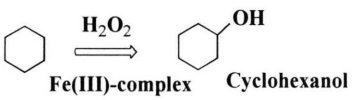
It is well known that some binuclear iron(III) compounds with μ-oxo bridge exhibit high catalase-like function, decomposition of hydrogen per-

oxide. We already reported that the diiron(III) compounds with μ-oxo bridge are divided into two classes, **A** and **B**, and class-**A** complexes exhibit high catalase-like function, whereas that of the class-**B** complexes, negligible as shown in Table II. The class-**B** compounds exhibit high activity for hydroxylation of cyclohexane in the presence of hydrogen peroxide, compared with that of class-**A** compounds. Here it is noteworthy that Fe(III) complex with (etapy) and (epy), which are considered to be a model for P-450 and its OMe-mutant, respectively, show high activity for hydroxylation of cyclohexane.

Table II. Turn-over numbers(TN) of cyclohexanol catalyzed by iron(III) compounds.

	Cyclohexanol 30 min	2 hours
Class-A complex		
Fe-(tpa)	0.06	0.3
Fe-(mopy)	0.3	0.8
Class-B complex		
Fe-(epy)	3.2	5.0
Fe-(etapy)	1.1	3.6
Fe-(tfpy)	1.2	4.6
Fe-(bbimae)[a]	1.3	6.0

[a] See ref. Takahashi *et al.* (1985); see Fig. 3 for abbreviations.



At present it is generally accepted that catalase-like function by the diiron(III) compounds may proceed through formation of an intermediate with μ-η^1:η^1-peroxide adduct(adduct-I), as shown in Fig. 5a (Ito *et al.*, 1996a; Menega *et al.*, 1994). This implies that formation of an another peroxide-adduct, whose structure is different from μ-η^1:η^1-coordination mode occurs in the solutions of compounds with (epy), (etapy) or (tfpy), and this should be an intrinsic active species for alkane functionalization. One of the possible structures in shown in Fig. 5b, adduct-II (Ito *et al.*, 1996a) where peroxide ion coordinates to an iron(III) ion in the (dpgs)-complex with η^1-coordination mode (Nishida *et al.*, 1995; Nishida and Ito, 1995a). In the peroxide adduct with η^1-coordination mode, both σ*- and π*-orbitals of the peroxide ion mix with d-orbital which directly interacts with the peroxide

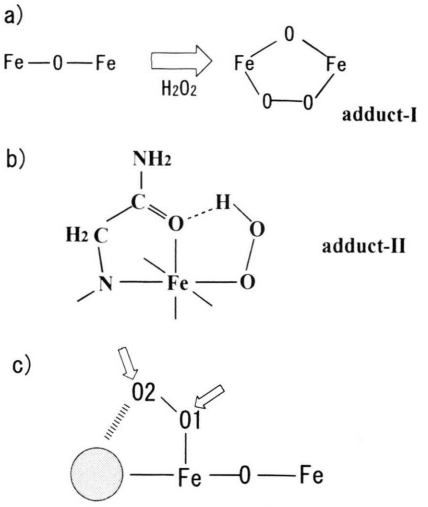


Fig. 5. Schematic illustrations of intermediates assumed in this paper.
a) (μ-η^1:η^1-peroxo)diiron(III);
b) η^1-coordination mode of peroxide ion;
c) reactive sites of the peroxide adduct with η^1-coordination mode.

ion (Ito *et al.*, 1996a). EHMO calculations revealed that the adduct-II shows electrophilic reactivity at both the O1 and O2 positions, as indicated by arrows in Fig. 5c (Nishida *et al.*, 1994; Nishida and Ito, 1995b).

The electrophilicity at O2 position has been confirmed by several experiments (Nishida and Ito, 1995b, Ito *et al.*, 1996b) especially in heme-oxygenase reaction (Torpey *et al.*, 1996). Thus, it has become apparent that interaction between O2 and ligand system of (dpgs)-ligand can contribute to the stabilization of adduct-II in energy. In Fig. 6, presence of orbitals A and B indicates that stabilization of the peroxide adduct occurs *via* interaction with the (epy)-ligand system, and also suggests that remarkable stabilization of energy of HOMO of (epy)-ligand, in this case p_z-orbital of ethereal oxygen, occurs through interaction with the peroxide ion; *this effect is specific for the ligand which contains a non-conjugated oxygen atom such as ethereal or alcoholic oxygen, for examples (epy), (etapy) and (tfpy).* Stabilization in energy as described for the (dpgs), (epy), (etapy) and (bbimae)-ligand systems is not detected for the cases of (tpa) and (mopy)-ligands, which may be due to both steric and electronic reasons (Ito *et al.*, 1996a). These are suggesting that electronic in-

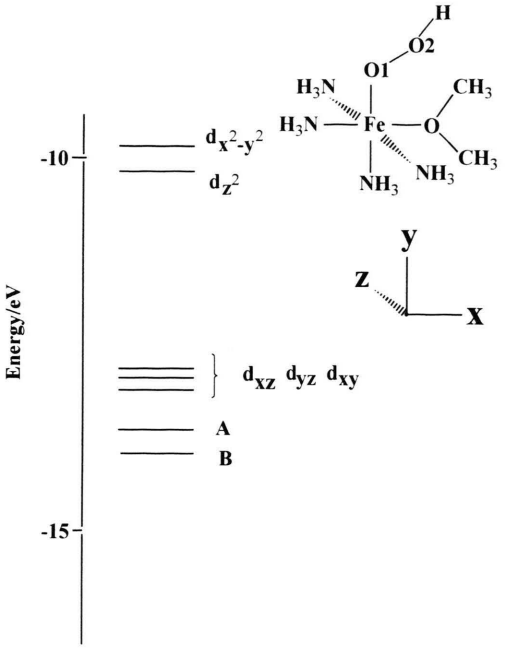


Fig. 6. EHMO calculation for $Fe(NH_3)_4(O_2H)(CH_3OCH_3)$ as a model for intermediate of OMe-mutant. (In the equations below, (C) denotes the carbon atom of methyl group close to the O2 atom; $p_x(C)$ and $p_z(O2)$ denote the p-orbitals of the carbon and oxygen atom, respectively; for details, see Hoffmann *et al.* (1977).

Orbital A: $-0.230p_x(C)+0.273p_y(C)+0.232p_x(O1)$
$-0.245p_y(O1)-0.388p_x(O2)+0.548p_y(O2)$

Orbital B: $0.529p_z(O)-0.263p_z(C)-0.389p_z(O1)$
$+0.604p_z(O2)$.

teraction between the peroxide in and organic moiety, which may originate from the electrophilic nature of d-orbital containing peroxide ion in the adduct-II, plays an important role to induce facile formation of adduct-II. In addition, it should be noted here that in the interaction between the d-orbital and the organic moiety, electron flows from the organic moiety to peroxide ion, and thus the peroxide ion coordinated to the iron(III) atom is more activated, because the electron flow to σ*-orbital of the peroxide occurs in this process (Ito *et al.*, 1996a).

If we assume that substrate approaches to the adduct-II, there should be electronic interaction between the HOMO of substrate and O1, since O1 atom of the adduct-II is also of electrophilic nature, as suggested in the papers (Nishida *et al.*, 1994; Ito *et al.*, 1996a). As the approach of substrate also donates electron to the adduct-II, the peroxide ion is more activated, leading to facile cleavage of O–O bond. There are two possible ways in the O–O bond in the peroxide adduct; i.e., homolytic and heterolytik cleavage; in the latter case, H_2O_2 is cleaved to OH_2 and a metal-O(atomic oxygen) (Schroder *et al.*, 1996; Bach and Su, 1994) and the latter species may be alternately formulated as a high-valent metal-oxo species. In the adduct-II, peroxide ion is unsymmetric, which may lead to facile heterolytic cleavage. Thus, activation of the peroxide ion through interactions with both the peripheral group and approach of substrate promote formation of a high-valent iron-oxo species, giving the oxygenated product (Bach *et al.*, 1995; Newcome *et al.*, 1995) as illustrated in Fig. 7a).

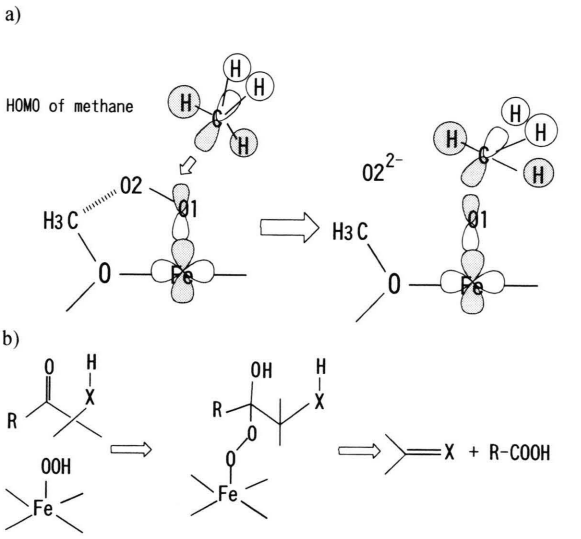


Fig. 7. a) Assumed scheme for reaction of methane with an Fe(III)-(epy)-peroxide adduct with η^1-coordination mode.
b) Reaction between (peroxo)iron(III) species with organic compounds containing carbonyl group.

This is meaning that interaction between the peroxide adduct and the peripheral organic group, and the approach of substrate play an important role in formation of an iron-oxo species; this consideration is completely different from that reported in the previous papers (see Fig. 1). Present discussions clearly explain several questions in P-450 reaction; that is, the bonding in the Thr252-dioxygen possesses important role in activation of peroxide ion, and may give an answer to the fact

that a compound I radical action perferryl oxygen species has not been identified for P-450's.

The importance of the electrophilicity at O2 atom of the peroxide adduct with η^1-coordination mode is also observed in several P-450's; aromatase and 14-demethylase catalyze not only the conventional hydroxylation reaction but also the oxidation of an alcohol into a carbonyl compound. These include a more important acyl-carbon bond cleavage reaction (see Fig. 7b)), (Vaz *et al.*, 1996; Pratt *et al.*, 1995) and degradation of amide-compounds (Ming *et al.*, 1995) and proteins (Rana and Meares, 1991).

It should be noted here that when target substrate contains an oxygen atom, the iron peroxide is trapped, producing an adduct which may decompose by one of several closely related pathways. These facts are also comprehensively explained by our model, i.e., when substrate contains an oxygen atom, similar to the ligand system of (epy), (tfpy) or (etapy), the electrophilic nature of O2 atom promotes the binding of O2 atom with the carbon atom of the substrate, which contributes to the stabilization of the system, and also to enhanced reactivity of the peroxide adduct, leading to facile hydroxylation or oxidation of the substrate. Our consideration may be applied to elucidate the reaction mechanism in non-heme oxygenases, such as in methane monooxygenase.

In the case of methane monooxygenase, it has been postulated that the intermediate Q reacts with methane directly, giving methanol (Lipscomb, 1994; Waller and Lipscomb, 1996). Until now, many reports have been published on the structure of the intermediate Q (Liu *et al.*, 1995). Very recently Que *et al.* have proposed that this intermediate has an $Fe(IV)_2O_2$ diamond core structure (see Fig. 8a)), which seems to be consistent with

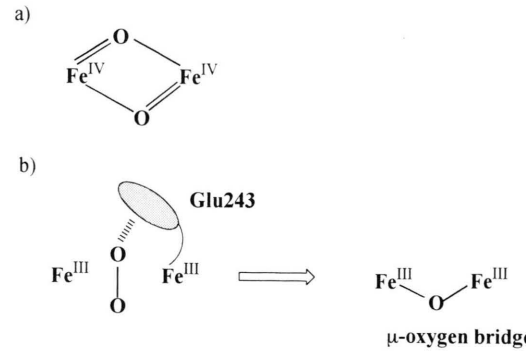


Fig. 8. a) An $Fe^{IV}_2O_2$ diamond core assumed by Que *et al.* for intermediate Q (Shu *et al.*, 1977).
b) Assumed scheme for formation of Fe(IV) state promoted *via* interaction between (peroxo)diiron(III) species and carboxylate group.

the results of Mossbauer Spectra and EXAFS data (Shu *et al.*, 1997). They have considered that the $Fe(IV)_2O_2$ diamond core forms *via* a $Fe(III)_2$-peroxide adduct, but this may be inconsistent with the recent result on the Ribonucleotide reductase, where formation of a Fe(IV) state is promoted by donation of one electron (Sturgeon *et al.*, 1996). Thus, it seems most likely that the interaction between the peroxide adduct and peripheral carboxylate group (Glutamate 243) (Feig and Lippard, 1994) should enhance heterolytic cleavage of the peroxide adduct(intermediate P), leading a Fe(III)-O(atomic oxygen)-Fe(III) species (see Fig. 8 b)), which can be written as a Fe(IV)-O(oxo)-Fe(IV).

Bach R. D., Mintcheva I., Esteva C. M. and Schlegel H. B. (1995), Theoretical model for an alternative mechanism for the cytochrome P-450 hydroxylation of quadricyclane. J. Am. Chem. Soc. **117,** 10121–10122.

Bach R. D. and Su M.-D. (1994), The transition state for the hydroxylation of saturated hydrocarbons with hydroperoxonium ion. J. Am. Chem. Soc. **116,** 10103–10109.

Feig A. L. and Lippard S. J. (1994), Reactions of nonheme iron(II) centers with dioxygen in biology and chemistry. Chem. Rev. **94,** 759–805.

Groves A. J. and Nemo T. E. (1983), Epoxidation reactions catalyzed by iron porphyrins. Oxygen transfer from iodosylbenzene. J. Am. Chem. Soc. **105,** 5786–5791.

Gunter M. J. and Turner P. (1991), Metalloporphyrins as models for cytochromes P-450. Coord. Chem. Rev. **108,** 115–161.

Hill C. L. (1989), Activation and Functionalization of Alkanes. John-Wiley & Sons, New York.

Harris D. L. and Loew G. H. (1994), A role for Thr 252 in cytochrome $P\text{-}450_{cam}$ oxygen activation. J. Am. Chem. Soc. **116,** 11671–11674.

Hoffmann R., Chen M. M.-L. and Thorn D. L. (1977), Qualitative discussion of alternative coordination modes of diatomic ligands in transition metal complexes. Inorg. Chem. **16,** 503–511.

Imai Y., Shimada H., Watanabe Y., Matsushima-Hibiya Y., Makino R., Koga H., Horiuchi T. and Ishimura Y. (1989), Uncoupling of the cytochrome $P450_{cam}$ monooxygenase activity by a single mutation, threonine-252 to alanine or valine: possible role of the hydroxy amino acid in oxygen activation. Proc. Natl. Acad. Sci. U.S.A. **86,** 7823–7827.

Ito S., Okuno T., Matsushima H., Tokii T. and Nishida Y. (1996a), Chemical origin of high activity in oxygenation of cyclohexane by H_2O_2 catalyzed by dinuclear iron(III) complexes with amide-containing ligands. J. Chem. Soc., Dalton Trans. 4479–4484.

Ito S., Suzuki M., Kobayashi T., Ohba S. and Nishida Y. (1996b), Formation and reactivity of a peroxide adduct of iron(III) complexes containing substituted phenol derivatives. J. Chem. Soc., Dalton Trans. 2579–2580.

Kimata Y., Shimada H., Hirose T. and Ishimura Y. (1995), Role of Thr-252 in cytochrome P-450CAM; a study with unnatural amino acid mutagenesis. Biochem. Biophys. Res. Commun. **208,** 96–102.

Kojima T., Leising R. A., Yan S. and Que L. jr. (1993), Alkane functionalization at nonheme iron centers; stoichiometric transfer of metal-bound ligand to alkane. J. Am. Chem. Soc. **115,** 11328–11335.

Leising R. A., Kim J., Perez M. A. and Que L., Jr. (1993), Alkane functionalization at (μ-oxy)diiron(III) centers. J. Am. Chem. Soc. **115,** 9524–9530.

Lipscomb J. D. (1994), Biochemistry of the soluble methane monooxygenase. Annu. Rev. Microbiol. **48,** 371–399.

Liu K. E., Valentine A. M., Wang D., Huynh B. H., Edmondson D. E., Salifoglou A. and Lippard S. J. (1995), Kinetic and spectroscopic characterization of intermediates and component interaction in reactions of methane monooxygenase from *Methylcoccus capsulatus.* J. Am. Chem. Soc. **117,** 10174–10185.

Menega S., Vincent J. M., Lambeaux C. and Fontecave M. J. (1994), μ-Oxo-bridged diiron(III) complexes and H_2O_2; monooxygenase- and catalase-like activities. J. Chem. Soc., Dalton Trans. 2081–2084.

Montellano P. R. (1986), Cytochrome P-450. Plenum Press, New York.

Newcomb M., Tadic M.-H., Putt D. M. and Hollenberg P. E. (1995), A nonsynchronous concerted mechanism for cytochrome P-450 catalyzed hydroxylation. J. Am. Chem. Soc. **117,** 12985–12091.

Nishida Y., Itoh H. and Yamazaki A. (1994), On the chemical mechanism of aldehyde metabolism by cytochrome P-450. Polyhedron **13,** 2473–2476.

Nishida Y., Okuno T., Ito S., Ohba S., Matsushima H. and Tokii T. (1995), Important role of tetrahydrofuran ring in activation of hydrogen peroxide in the presence of binuclear iron(III) complexes with linear μ-oxo bridge. Chem. Lett. 885–886.

Nishida Y. and Ito S. (1995a), High activity of an Fe-tfda complex for hydroxylation at the aromatic and alkane rings of 2′-deoxyguanosine in the presence of hydrogen peroxide. J. Chem. Soc., Chem. Commun. 1211–1212.

Nishida Y. and Ito S. (1995b), Comparison on reactivity of Fe(III) and Al(I) compounds in the presence of hydrogen peroxide; its relevance to possible origin for central nervous system by aluminium ion. Z. Naturforsch. **50c,** 571–577.

Peng, H-Ming, Raner G. M., Vaz A. D. N. and Coon M. J. (1995), Oxidative cleavage of esters and amides to carbonyl compounds by cytochrome P-450. Arch. Biochem. Biophys. **318,** 333–339.

Poulas T. L., Finzel B. C. and Howard A. J. (1987), High-resolution of crystal structure of cytochrome $P\text{-}450_{CAM}$. J. Mol. Biol. **195,** 687–700.

Pratt J. M., Ridd T. I. and King L. J. (1995), Activation of H_2O_2 by P450; Evidence that the hydroxylation intermediate is iron(III)-coordinated H_2O_2 and not the ferryl FeO^{3+} complex. J. Chem. Soc., Chem. Commun. 2297–2298.

Raag R., Martinis S. A., Sligar S. G. and Poulas T. L. (1991), Crystal structure of the cytochrome $P\text{-}450_{CAM}$ active site mutant Thr252Ala. Biochemistry **30,** 11420–11429.

Rana T. M. and Meares C. F. (1991), Transfer of oxygen from an artificial protease to peptide carbon during proteolysis. Proc. Natl. Acad. Sci. USA **88,** 10578–10582.

Schroder D., Schalley C. A., Goldberg N., Hrusak J. and Schwarz H. (1996), Gas-phase experiments aimed at probing the existence of the elusive water oxide molecule. Chem. Eur. J. **2,** 1235–1242.

Shu L., Nesheim J. C., Kauffmann K., Munck E., Lipscomb J. D. and Que L. jr. (1997), An $Fe^{IV}_2O_2$ diamond core structure for the key intermediate Q of methane monooxygenase. Science **275,** 515–518.

Sono M., Roach M. P., Coulter E. D. and Dawson J. H. (1996), Heme-containing oxygenases. Chem. Rev. **96,** 2841–2887.

Sturgeon B. E., Burdi D., Chen S., Huynh B.-H., Edmondson D. E., Stubbe J. and Hoffman B. M. (1996), Reconsideration of X, the diiron intermediate formed during cofactor assembly in *E. coli* ribonucleotide reductase. J. Am. Chem. Soc. **118,** 7551–7557.

Takahashi K., Nishida Y., Maeda Y. and Kida S. (1985), Crystal structures and magnetism of binuclear iron(III) complexes with a linear oxo-bridge, $[Fe_2O(bbimae)_2X_2][NO_3]_2$. J. Chem. Soc., Dalton Trans. 2375–2380.

Torpey J., Lee D. A., Smith K. M. and Montellano P. R. (1996), Oxidation of an α-meso-methyl-substituted heme to an α-biliverdin by heme-oxygenase. A novel heme cleavage reaction. J. Am. Chem. Soc. **118,** 9172–9173.

Vaz A. D. N., Pernecky S. J., Raner G. M. and Coon M. J. (1996), Peroxo-iron and oxenoid species as alternative oxygenating agents in cytochrome P-450-catalyzed reactions. Switching by threonine-302 to alanine mutagenesis of cytochrome P-450 2B4. Proc. Natl. Acad. Sci. U.S.A. **93,** 4644–4648.

Waller B. J. and Lipscomb J. D. (1996), Dioxygen activation by enzymes containing binuclear non-heme iron clusters. Chem. Rev. **96,** 2625–2657.