Electronic Property and Reactivity of (Hydroperoxo)Metal Compounds

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(Hydroperoxo)Metal Compound, DFT Calculation, Oxygenase Reaction

DFT calculations were done for the (hydroperoxo)metal complexes with η^1-coordination mode, where metal ions are Fe(III), Al(III), Cu(II) and Zn(II). Results shows that 1) the electron density at the two oxygen atoms of the hydroperoxide ion is highly dependent on the angle O-O-H in M-OOH species and the difference in electron density between the two oxygen atoms reaches a maximum at the angle O-O-H = 180°, 2) total electron density at the two oxygen atoms of the peroxide ion increases by approach of methane to the (hydroperoxo)metal species in the cases of Fe(III) and Cu(II); on the other hand, significant decrease of the electron density on peroxide oxygen atoms was observed for the cases of Al(III) and Zn(II) compounds. These findings suggest that the (hydroperoxo)metal species acts as an electrophile in the former cases (M = Fe(III), Cu(II)) and as a nucleophile for the latter two compounds (M = Zn(II), Al(III)). The electrophilicity observed for the Fe(III) and Cu(II) complexes is attributed to the presence of unoccupied- or half-filled d-orbitals interacting with the hydroperoxide ion. 3) Two oxygen atoms of the (hydroperoxo)-compounds of Fe(III) and Cu(II) complexes exhibit quite different reactivity toward the substrate, such as methane. When methane approaches the oxygen atom which is coordinated to a metal ion, a strong decrease of electron density at the methane carbon atom occurs with concomitant increase of electron density at the peroxide oxygen atoms inducing its heterolytic O-O cleavage. When methane approaches the terminal oxygen atom, an oxidative coupling reaction occurs between peroxide ion and methane; at first a nucleophilic attack by the terminal electron-rich oxygen atom occurs at the carbon atom to induce C-O bond formation, and a subsequent oxidative electron transfer proceeds from substrate to the metal-peroxide species yielding CH_3-OOH, CH_3OH, or other oxidized products. These results clearly demonstrate that the (hydroperoxo)-metal compound itself is a rather stable compound, and activation of the peroxide ion is induced by interaction with the substrate, and the products obtained by the oxygenation reaction are dependent on the chemical property of the substrate, redox property of a metal ion, and stability of the compounds formed in the intermediate process.

Introduction

As is well-known, a (hydroperoxo)metal species plays an very important role in many biological oxygenases. (Sono *et al.,* 1996; Klinman, 1996; Crane *et al.,* 2000; Newcomb *et al.,* 2000) In Fig. 1 (A), the reaction mechanism for cytochrome P–450 advocated by Groves and Nemo is illustrated. (Groves and Nemo, 1983) Model systems have shown that a Fe(V) = O, ferryl oxo-species reacts with an organic compound to give an oxygenated product, and Raman spectroscopy studies have supported the process of oxygenation in Fig. 1 (A), however high-valent iron-oxo species have never been detected in the biological reaction cycle until now (Schlichting *et al.,* 2000). The most crucial problem in this mechanism is that heterolytic O-O cleavage is induced by the proton, to induce the formation of high-valent iron-oxo species, and this assumption is inconsistent with the usual chemical sense, because it is well known that the O-O bond of the peroxide ion cleaves only when another electron is introduced to σ*-orbital of the peroxide ion (Nishida, 1998).

We have proposed the new idea to elucidate the reaction mechanisms of biological oxygenases, including cytochrome P450 (see Fig. 1 (B)); in our idea we have pointed out the importance of electrophilicity of a (hydroperoxo)metal species (metal = Fe(III) and Cu(II)), and proposed that O-O heterolysis of the peroxide ion is induced by introducing the electron from HOMO of substrate into the σ*-orbital of the peroxide ion, and we are considering that the metal-peroxide adduct is only

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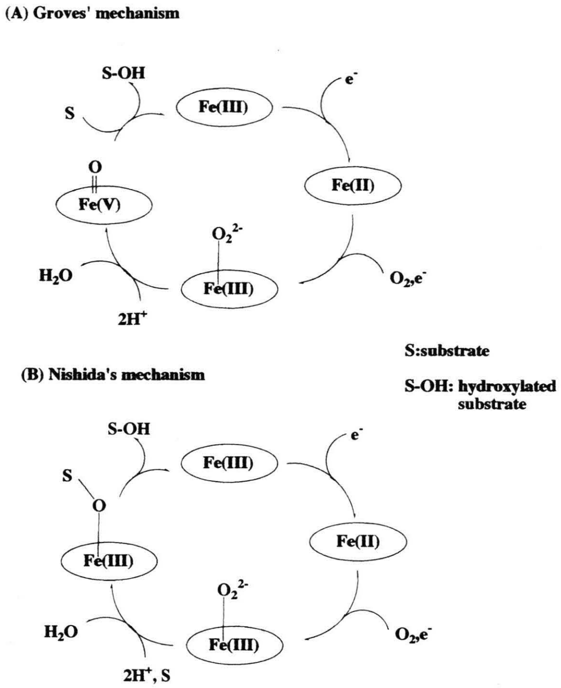


Fig. 1. Chemical mechanism in cyochrome P-450 proposed by (A) Groves and Nemo (1983) and (B) Nishida.

an intermediate, and it is activated through interaction with the substrate, and the activation is dependent on the chemical property of the substrate, and mode of interaction (Nishida, 1998). In some cases, presence of the substrate may lead to formation of a metal-peroxide adduct, which has been exemplified in some model systems (Nishino *et al.,* 1999b).

Recently, several papers have been published to support that a metal-peroxide adduct acts as an electrophile (Ito *et al.,* 1998; Montellano, 1998; Newcomb *et al.,* 2000), but detailed theoretical investigations on the electronic property of metal-hydroperoxide adducts seem quite scarce. Very recently, the density-functional theory (DFT) (Parr and Young, 1989) has emerged as an accurate alternative first-principles approach to quantum mechanical molecular investigations. DFT currently accounts for approximately 90% of all quantum chemical calculations being performed, not only because of its proven chemical accuracy, but also because of its relatively cheap computational expense (Basch *et al.,* 1999; Yoshizawa *et al.,* 1998; Godbout *et al.,* 1999; Patchkovskii and Ziegle, 2000; Dunietz *et al.,* 2000; Ceccarelli *et al.,* 2000). In this study we have investigated the electronic property and reactivity of a (hydroperoxo)metal species by the DFT method. The calculations were performed using DGauss 4.1 (Oxford Molecular Science, Oxford 1998) and Q-Chem (Q-Chem. Inc., Pittsburgh, 1998).

Calculations

DFT calculations were done as follows (Parr and Young, 1989):
method I, DFT by DGauss 4.1. basis set, DZVP, Gradient GGA X B88; GGA C LYP 88;
method II, DFT by DGauss 4.1; basis set DZVP, Gradient GGA X B88, GGA C PW91;
method III, DFT by Q-Chem, basis set, 3–21G, hybrid LYP88.

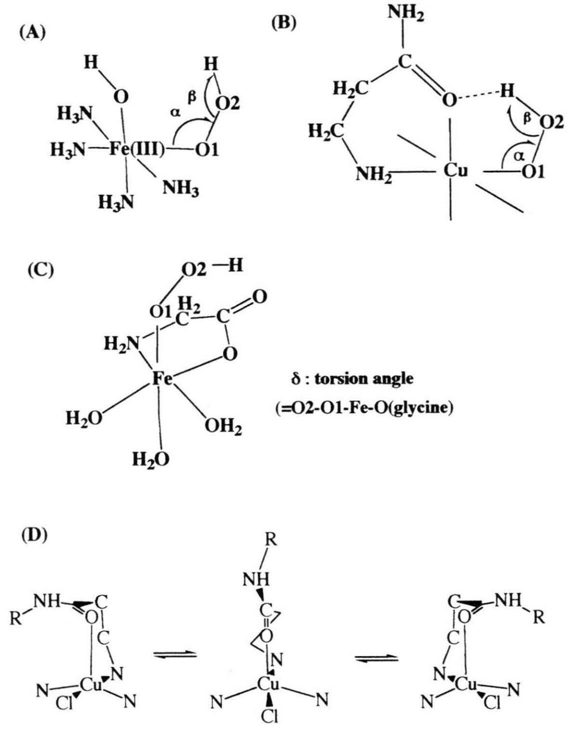
Structural features of the compounds were based on the crystal structure determination of the analogous compounds. Supplementary Data on DFT calculations are available from the authors.

Results

(Hydroperoxo)iron(III) compounds

We have compared the calculated results by using several methods (method I, II, and III) for the same compounds; $Fe(NH_3)_4(OH)(OOH)$, (see Fig. 2 (A)) $Fe(OH_2)_3(glycinato)(OOH)$, (see Fig. 2 (C)) and the corresponding Al(III) compounds were examined. For $Fe(NH_3)_4(OH)(OOH)$ the structure is characterized by two parameters, α and β, as illustrated in Fig. 2 (A).

Method I, II, and III give almost the same calculated results on electron densities of the atoms in molecule. This is an important finding. For example, change in electron density at oxygen atoms of the peroxide ion with angle β are shown in Fig. 3 (A) and (B), which were obtained for $Fe(NH_3)_4(OH)(OOH)$ by method I and method III, respectively. It should be noted here that electron density at the oxygen atoms of the peroxide ion is highly dependent on the position of the proton atom (angle β), and similar feature was also detected for the corresponding Al(III) compound, $Al(NH_3)_4(OH)(OOH)$ (Supplementary Data). The electron density of the terminal oxygen atom, O2 increases with increasing angle β, and reaches its highest value at $\beta = 180°$,



δ : torsion angle (=O2-O1-Fe-O(glycine)

Fig. 2. Structural features of compounds examined in this paper.
(A) $Fe(NH_3)_4(OH)$ (OOH), (B) Cu(β-alanineamide)-$(H_2O)_2$(OOH),
(C) Fe(glycinato)$(OH_2)_3$(OOH), (D) Three possible geometrical isomers of compound (B).

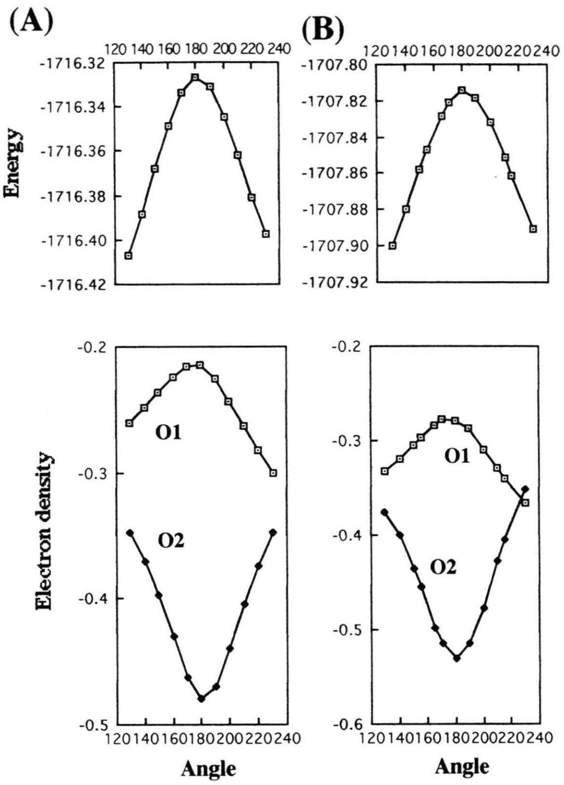


Fig. 3. Upper, energy change (Eh), lower; electron density change at oxygen atoms of the peroxide ion with the angle β (O1-O2-H) in $Fe(NH_3)_4(OH)$ (OOH) calculated by method I and method III for (A) and (B), respectively.

and decreases in the range 180–230°. At β = 180° the hydrogen atom of the hydroperoxide ion is most positively charged (Supplementary Data), indicating that the O-H bond is more unstable at this stage, and the electron density on iron(III) atom remains unchanged throughout this structural change (Supplementary Data). Energetically the state with β = 130 or 230° may be the most stable state (see the change of total energy with the angle δ in Fig. 3), but other states such as β = 170 ~ 190° may occur during the reaction through interaction with the substrate; under these conditions (states with β = 170 ~ 190°) it seems quite likely that O-O heterolysis ($O_2^{2-} \rightarrow O$ and O^{2-}) of the peroxide ion occurs readily because the electron density difference between the O1 and O2 is quite big.

The calculated results obtained for $Fe(OH_2)_3$(OOH)(glycinate) were deposited as Supplementary Data. In this case the interaction between the hydroperoxide ion and the organic part of the ligand may occur, and the results show that the electron density at oxygen atom is dependent on the angles, α, β, and δ (O2-O1-Fe-O (glycine molecule) (see Supplementary Data). With increasing angle of δ (from 0° to 30°), the terminal oxygen atom O2 approaches the C-H bond of the glycine molecule, and electronic interaction may occur between them. This is reflected by the higher electron density at the terminal oxygen atom O2. It should be noted that the electron density change at oxygen atoms is not continuous in the system α = 120, β = 130~230°, and δ = 60. Similar phenomena are more frequently observed in the system α = 110, and δ = 65° (see Supplementary Data). In the case of Al(III) complex, such a discontinuous change was not observed for the same compound. This suggests that interaction between the C-H bond of the glycine molecule and terminal oxygen atom O2 of the peroxide atom occurs in the Fe(III) complex. This problem will be discussed again in the next section.

Copper(II)-(hydroperoxo) compound

In our previous papers, we have reported that the Cu(bdpg)ClO_4 complex exhibits reactivity towards several substrates different from that of Cu(tpa)ClClO_4 complex, and pointed that the unique reactivity observed for the former compound should be attributed to the facile formation of a hydroperoxide adduct through hydrogen bonding with the ligand system as shown in Fig. 2 (B) (Okuno *et al.,* 1997; Kobayashi *et al.,* 1998).

To support the previous proposition, DFT calculations were made for this system. As shown in our previous paper (Nishino *et al.,* 2000), there are several conformational isomers for the present copper(II) complex (see the Fig. 2 (D): linear-structure (central) and bent-structure (right and left). The calculated results for the linear-structure in Fig. 2 (D) (R = H) are illustrated in Fig. 4 (A). The electron densities at the oxygen atoms are highly dependent on the angle α (in this case, β was set at 120°), and the difference between the electron density of the two oxygen atoms becomes larger with the decreasing of α. At this time, the electron density at the oxygen atom of amide-carboxylate also increases with decreasing α. Of course, the electron density at the oxygen atoms are highly dependent on the angle β, as shown in Fig. 4 (C) (in this case α was set to be 110°). In the bent structure, the increase on the electron density of O2 atom is larger than that observed for the linear-structure (Fig. 4 (B)), which is attributed to the more stronger interaction between the O2 atom and organic group of the ligand system.

Reaction between the (hydroperoxo)metal species and substrate

For more information on the reactivity of the (hydroperoxo)metal species, we have calculated the electron density change of the compound when methane approaches the system. There are several ways in which methane approaches to the metal compounds, and thus we have done the calculations on the assumption that 1) methane approaches the peroxide adduct in two ways (see Fig. 5), case (A) and case (B); where methane approaches the oxygen atom, O1 and O2, respectively, and 2) methane approaches the peroxide adduct where HOMO of the methane molecule (Fleming, 1976) interacts with the oxygen atom of the peroxide adduct. Similar calculations were also

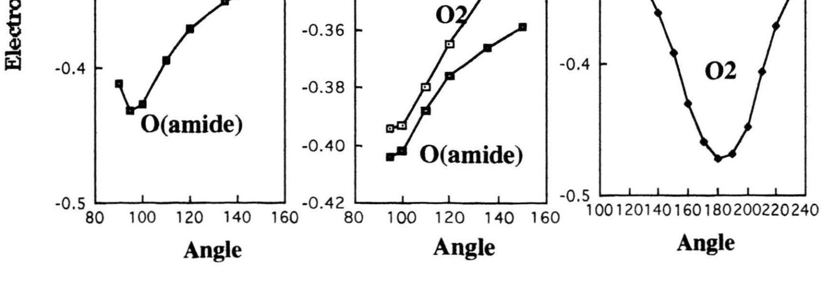


Fig. 4. Electron density change at the oxygen atoms of copper(II) species with angle α
(A) linear-structure and (B) bent structure obtained by method I, (β = 120°).
(C) Electron density change at oxygen atoms of the peroxide ion with angle β in copper(II) complex with linear structure calculated by method I where α = 110.

done for the copper(II) complex (see Fig. 5 (B)), and the results were listed in Table II.

The calculated results obtained for the iron(III) compounds are summarized as follows; i) the electron density at the oxygen atoms of the peroxide ion changes with the approach of methane molecule, which is highly dependent as to how the methane molecule approaches O1 or O2 atom of the peroxide ion. In case (A) of Fig. 5 (A), the reduction of electron density at the carbon atom of methane, the increase of total electron density at the peroxide oxygen atoms, and the electron density reduction on the iron atom occur (see Table I). These findings suggest that in case (A), the electron is transferred from the HOMO of methane to the peroxide ion, leading to O-O heterolysis, to give methanol *via* an intermediate complex formation containing a species similar to Fe(V)-oxo species as illustrated in Fig. 6 (I). Above behavior is also dependent on angle β, but its effect is rather small.

ii) In case (B), the increase of electron density at the carbon atom of methane is observed when methane approaches the O2 atom, and electron density decrease at O2 atom is notable at β = 160°, but total electron density at both two oxygen atoms of the peroxide ion increases with the approach of methane; this is quite different from those observed for the Al(III) and Zn(II) compounds (Nishino *et al.*, submitted). These imply that a Fe(III)-hydroperoxide adduct acts as an electrophile toward methane and other organic compounds. This behavior is highly dependent on angle β, and it is remarkable for the state with β = 170 ~ 190°. This should be attributed to the high electron density at the O2 atom, and above behavior is closely related to the high nucleophilicity of the O2 atom.

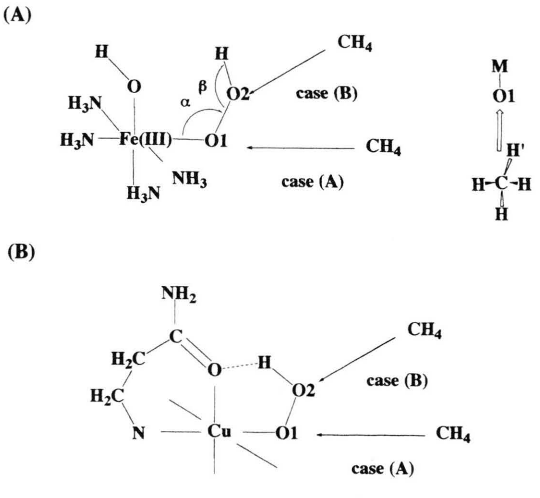


Fig. 5. Approach of methane to a (hydroperoxo)metal compound.

Table I. Calculated Mulliken's net atomic charge at atoms where methane molecule is approaching (A) $Fe(NH_3)_4(OH)(OOH)$ and (B) $Al(NH_3)_4(OH)(OOH)$. *R* denotes the distance between C (methane) and oxygen atom.

Atom	*R* [nm]=18	=20	=30	=40
(A) Fe(III) complex				
case (A), β=120				
Fe	0.808	0.803	0.751	0.739
O1	−0.379	−0.352	−0.281	−0.271
O2	−0.402	−0.372	−0.336	−0.329
C(methane)	−0.723	−0.744	−0.809	−0.817
case (A), β=160				
Fe	0.810	0.805	0.756	0.745
O1	−0.344	−0.308	−0.235	−0.224
O2	−0.460	−0.442	−0.428	−0.428
C(methane)	−0.713	−0.744	−0.813	−0.820
case (B), β=120				
Fe	0.733	0.738	0.737	0.737
O1	−0.328	−0.288	−0.268	−0.270
O2	−0.359	−0.36	−0.332	−0.329
C(methane)	−0.829	−0.816	−0.819	−0.820
case (B), β=160				
Fe	0.736	0.740	0.743	0.743
O1	−0.328	−0.274	−0.225	−0.224
O2	−0.357	−0.404	−0.427	−0.429
C(methane)	−0.931	−0.896	−0.837	−0.825
(B) Al(III) Complex				
case (A), β=120				
Al		0.892	0.865	0.858
O1		−0.451	−0.432	−0.427
O2		−0.434	−0.445	−0.442
C(methane)		−0.768	−0.815	−0.817
case (A), β=160				
Al		0.872	0.853	0.846
O1		−0.38	−0.358	−0.352
O2		−0.493	−0.524	−0.527
C(methane)		−0.765	−0.818	−0.820
case (B), β=120				
Al		0.840	0.857	0.858
O1		−0.436	−0.424	−0.426
O2		−0.436	−0.441	−0.442
C(methane)		−0.838	−0.821	−0.819
case (B), β=160				
Al		0.819	0.843	0.845
O1		−0.392	−0.352	−0.352
O2		−0.464	−0.522	−0.528
C(methane)		−0.915	−0.839	−0.824

The nucleophilic attack by the O2 to the carbon atom may induce formation of a chemical bond between carbon atom and O2 atom, but in this case a subsequent electron withdrawing process from substrate is necessary to complete the forma-

Table II. Calculated Mulliken's net atomic charge at atoms where methane is approaching copper(II)-peroxide system in Fig. 2 (B) (linear structure). *R* denotes distance between C (methane) and oxygen atom.

Atom	*R* [nm]=18	=20	=30
case (A), β=120			
Cu	0.554	0.575	0.533
O1	−0.352	−0.359	−0.258
O2	−0.438	−0.441	−0.334
H(-OOH)	0.432	0.433	0.442
C(methane)	−0.731	−0.744	−0.809
H(methane)	0.276	0.253	0.199
	0.231	0.217	0.199
	0.277	0.253	0.207
	0.196	0.196	0.202
case (B), β=120			
Cu	0.514	0.519	0.520
O1	−0.347	−0.287	−0.245
O2	−0.391	−0.383	−0.329
H(-OOH)	0.457	0.445	0.434
C(methane)	−0.829	−0.811	−0.815
H(methane)	0.267	0.237	0.199
	0.201	0.191	0.195
	0.294	0.249	0.209
	0.248	0.231	0.216
case (B), β=160			
Cu	0.53	0.545	0.565
O1	−0.325	−0.284	−0.229
O2	−0.366	−0.411	−0.432
H(-OOH)	0.414	0.434	0.456
C(methane)	−0.862	−0.838	−0.818
H(methane)	0.271	0.244	0.196
	0.224	0.210	0.200
	0.281	0.251	0.204
	0.265	0.248	0.219

tion of C-O bond (see Fig. 6 (II)) (Plesnicar, 1983). If an electron acceptor is a metal-peroxide adduct, the hydroxylated product CH_3OH may form by concomitant heterolytic O-O bond cleavage (case (a)). If an electron acceptor is a metal ion, there may be formation of an organic-hydroperoxide (case (b)). In some cases, the electron transfer may lead to the degradation of the substrate, as shown in case (c) in Fig. 6 (II) (Nishino *et al.,* 2000; Sasaki *et al.,* 1999). We must recall that electron density change of the peroxide ion is discontinuous in the case $Fe(OH_2)_3$(glycinate)(OOH) when β is in the range 160–190°. This may support the occurrence of case (II) in Fig. 6.

iii) In case (B), reduction in electron density at O2 and its increase at the carbon atom were observed for the Al(III) and Zn(II) compounds, which is similar to the cases observed for Fe(III) and copper(II) as described above. However in the former cases the total electron density at the two peroxide oxygen atoms is reduced with the approach of methane molecule (see Table I). This is indicates that the hydroperoxo species of Al(III) and Zn(II) compounds acts as a nucleophile towards the organic compounds, and the electrophilicity of the peroxide ion is strongly depressed through the coordination to a metal ion in Al(III) and Zn(II) compounds.

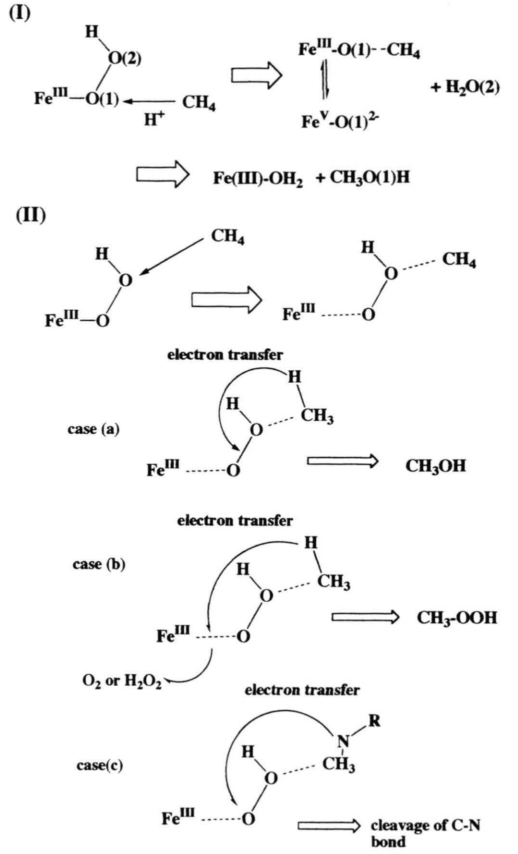


Fig. 6. Reaction pathway assumed for (hydroperoxo)-metal compound and organic molecule.

Experimental and Calculated Results

In our previous papers, we have reported that Al(III) exhibits quite different reactivity from that of Fe(III) in the presence of hydrogen peroxide, for example Fe(III) can hydroxylate the benzene ring in the presence of hydrogen peroxide, but

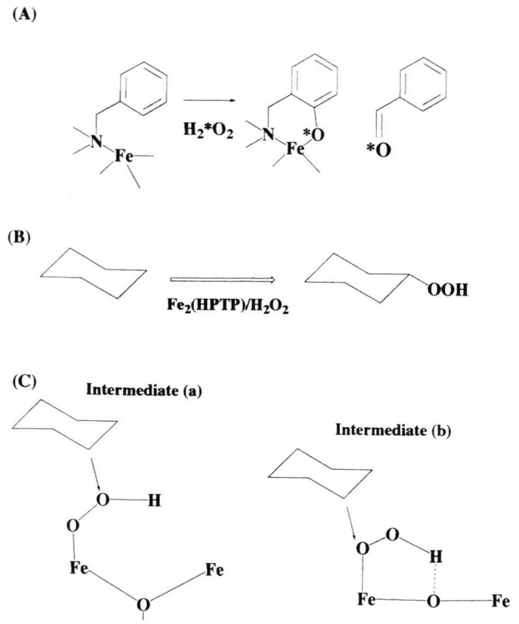


Fig. 7. (A) Reaction products from Fe(III)-(benzylamine) system and hydrogen peroxide.
(B) Reaction product in $Fe_2(HPTP)/H_2O_2$ and cyclohexane system.
(C) Tow possible reaction pathways assumed for Fe(III)-(hydroperoxide) and cyclohexane.

Al(III) ion cannot (see Fig. 7 (A)) (Nishida and Ito, 1995). We have pointed out the importance of the presence of unoccupied or half-occupied d-orbital which can interact with the hydrogen peroxide for the unique reactivity of the iron(III) compounds, and our proposition is clearly supported by the present calculation as described above. We have measured the ESI-mass spectra of the solution containing Al(III) and (bzda); (H_2(bzda) = benzylamine- N,N-diacetic acid (Nishida and Ito, 1995)) (Supplementary Data). In the spectra of Al(III) species, there are several peaks at m/z = 266.0, 321.0, 339.0, 513.0 and 531.0, which can be assigned to the species, $[Al(bzda)(H_2O)]^+$, $[Al(Hbzda)(H_2O)_4]^+$, $[Al(Hbzda)(H_2O)_5]^+$, $[Al(bzda)\text{-}(OH)\text{-}Al(bzda)]^+$, and $[Al_2(bzda)_2(OH)(H_2O)]^+$, respectively. No change was observed in the spectrum by the addition of hydrogen peroxide to this solution. When hydrogen peroxide was added to the iron(III) complex solution, the mono-oxygenated species of H_2bzda, originally observed at m/z = 224.2 was detected at m/z = 240.2 (which shifts to m/z = 242.2 when $H_2{}^{18}O_2$ was added). In addition to this, we have detected new signal at m/z = 107.0 (which shifted to m/z = 109.0 when when $H_2{}^{18}O_2$ was used), and this should be due to the formation of benzaldehyde, as illustrated in Fig. 7 (A). These features are not observed for the case of Al(III) complexes, and indicates that the electrophilic property of hydrogen peroxide is totally depressed in the solution containing Al(III) ion, which is consistent with the calculated results. The formation of benzaldehyde should be derived from the oxidative C-N bond cleavage according to case (c) in Fig. 6 (II).

Previously, we have reported that cyclohexane-hydroperoxide and hydroxy-cyclohexane were obtained from the reaction mixture containing iron(III) complex, hydrogen peroxide, and cyclohexane, (see Fig. 7 (B)) and pointed out that binuclear iron(III) complex, $Fe_2(HPTP)Cl_4{}^+$ selectively gives cyclohexane-hydroperoxide (Nishino *et al.,* 1999a). We have proposed the following mechanism: cyclohexanol is obtained through interaction at the O1 atom (intermediate (b) in Fig. 7 (C))), and cyclohexane hydroperoxide, with O2 atom (intermediate (a) in Fig. 7 (C)), respectively and this is consistent with the calculated results obtained in this study.

The formation of a hydroperoxo-iron(III) intermediate has been assumed for both cytochrome P450 and heme-oxygenase enzymes (Sono *et al.,* 1996; Montellano 1998). We have calculated the electron density at the oxygen atoms in Fe(III)-(OOH)(OM-por) species, where H_2(OM-por) represents octamethyl-porphyrin (Part of this work was already reported by Nishida (2000)).

The basic property of the porphyrin compound is essentially the same as those reported in this study (see Supplementary Data). Thus, it seems quite likely that the oxygenation reaction in cytochrome P–450 proceeds according to case (I) in Fig. 6, and our mechanism may be supported by the recent crystal structure determinations (Schlichting *et al.,* 2000); these authors have succeeded to detect an intermediate Fe-O species only when the substrate is present. In the case of heme-oxygenase, the oxygenation reaction may proceed according to case (a) in Fig. 6 (II): in this case the nucleophilic attack of the peroxide ion to the carbon atom of the porphyrin ring occurs, and the subsequent heterolytic O-O cleavage of the peroxide adduct yields hydroxylated porphyrin.

We have observed that $Cu(tpa)ClClO_4$ complex exhibits quite different reactivity from that of the $Cu(bdpg)ClClO_4$, and this difference has been attributed to the difference in reactivity of a peroxo-copper(II) species formed during the reaction (Okuno *et al.,* 1997; Kobayashi *et al.,* 1998). As described above, in the case of $Cu(bdpg)Cl^+$ complex, the structure of a peroxo species is undoubtedly illustrated by Fig. 2 (B). But, in case of $Cu(tpa)(OOH)^+$, β should be in the range 170~200, because this complex has no organic group which interact with a hydroperoxo group through hydrogen bonding. It is quite likely that the nucleophilic attack by the O2 atom is much stronger in the (tpa) complex than that in (bdpg) complex (see Fig. 4 (C)).

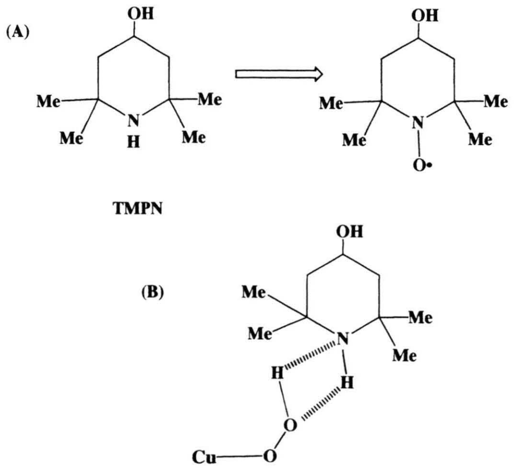


Fig. 8. (A) Formation of the nitrone radical of TMPN. (B) Assumed reaction pathway between Cu(tpa)(OOH) and TMPN.

We have reported that the $Cu(tpa)/H_2O_2$ system gives a nitrone radical of 2,2,6,6,- tetramethyl–4-piperidinol, (see Fig. 8 (A)), but the $Cu(bdpg)/H_2O_2$ system, does not (Nishino *et al.,* 1999b). It should be noted that TMPN is one of the spin-trapping reagents for singlet oxygen ($^1O_2$). DFT calculation for TMPN have revealed that HOMO of this compound is mainly developed on N-H bond (see Supplementary Data). As this compound reacts rapidly with singlet oxygen, an electrophile, it seems likely that TMPN attacks an electrophilic (peroxo)copper(II) species as shown in Fig. 8 (B): In this case, the highly negative, electron-rich O2 atom and positive H atom of the (peroxo)copper(II) species may promote the approach of these compound and electron transfer between them. The hydrogen-bonded species of Cu(bdpg)(OOH) seems unfavorable for this reaction. It is alarming to learn that we can detect the nitrone radical of TMPN even though there is no singlet oxygen in the solution. This suggests that TMPN, one of the substrates, activates the peroxide as if the (peroxo)copper(II) species behaves as singlet oxygen ($^1O_2$). We have reported that DPMO-OH is detected in the solution containing binuclear iron(III) complex, $Fe_2(HPTP)(OH)(NO_3)_4{}^+$, and hydrogen peroxide, where DPMO is a well-known spin-trapping reagent for OH• radical (Nishida *et al.,* 1992). It was proposed that in this system DMPO reacts facilely with an iron(III)-peroxide adduct, to promote the O-O cleavage, and the OH• formed reacts with DMPO to give DMPO-OH, which was detected by ESR spectrometry. Apparently DMPO is very reactive toward the peroxo-iron(III) species, and thus all discussions derived from the results obtained on "active oxygen species" in the biological system with spin-trapping reagents (Halliwell and Gutteridge, 1985) should be reconsidered.

The $Cu(tpa)Cl^+$ complex gives only a Form II DNA when reacting with Form I DNA in the presence of hydrogen peroxide, but highly degraded DNA is observed in the reaction mixture of Cu(bdpg)Cl, Form I DNA, and hydrogen peroxide. This may be due to the different reactivity of the peroxide adduct assumed for the both compounds (Nishino *et al.,* 1999b; Nishida, 1999). Very recently we have reported that marked release of nucleic acids was observed in the reaction mixture containing $Cu(bdpg)Cl_2$, oligonucleotides (12-mer, d(5′-CGCTTTAAAGCG) or 8-mer, d(5′-AAAGGTTT)) and hydrogen peroxide, but no release was detected in the solution containing $Cu(tpa)ClClO_4$ under the same experimental conditions (Nishida, 1999). The release of nucleic acids has been shown to be due to the formation of 4′-hydroxylated species, (Stubbe and Kozarich, 1987; Nishida, 1999) and thus the release of nucleic acid by the $Cu(bdpg)/H_2O_2$ system may proceed through the formation of 4′-hydroxylated species; the electrophilic nature of the peroxo-copper(II) species is very important for its formation.

As the nucleophilic ability of the terminal oxygen atom in $Cu(tpa)(OOH)^+$ is very strong, the DNA cleavage in this case may be attributed to a hydrolytic mechanism, i.e., Cu(tpa)(OOH) acts as a nucleophile toward the phosphate linkage of DNA, to cleave it. Hydrolytic DNA cleavage due to a nucleophilic metal peroxide adduct is sometimes observed; for example in the La(III)-peroxide system, (Takasaki and Chin, 1993) and binuclear zinc(II)-peroxide system (Nishino *et al.*, unpubl. results).

Supplementary data*

1. Calculated result for TMPN molecule by method III, 2. DFT calculation for Fe$(NH_3)_4$(OH)(OOH) by Method III, 3. Energy change (Eh) and electron density change at oxygen atoms of the peroxide ion with the angle β (O1-O2-H) in Al$(NH_3)_4$(OH)(OOH) calculated by method I, 4. Energy change (Eh) and electron density change at oxygen atoms of the peroxide ion with the angle β (O1-O2-H) in Al$(NH_3)_4$(OH)(OOH) calculated by method III, 5. Electron density change on iron and H (-OOH) atoms with angle β in Fe$(NH_3)_4$(OH)(OOH) calculated by method III, 6. Electron density change at oxygen atoms of the peroxide ion with angle β in Fe$(OH_2)_3$(glycinato)(OOH) calculated by method III where α = 120, δ = 60°, 7. Electron density change of (A) H(-OOH), (B) O1, (C) O2, (D) C (methylene carbon of glycine molecule) with angle β in Al$(OH_2)_3$(glycinato)-(OOH) calculated by method III, where α = 110, δ = 65°, 8. ESI-Mass spectrum of the solution containing Al(III)-(bzda) complex, and 9. ESI-Mass spectra of the solution containing Fe(III)-(bzda) solution.

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* Supplementary data on crystal structure determination, DFT calculations and ESI-mass spectra of the compounds are available from the author by requests.

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