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Water-Assisted Oxo Mechanism for Heme Metabolism

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Abstract: A mechanism of heme metabolism by heme oxygenase (HO) is discussed from B3LYP density functional theory calculations. The concerted OH group attack to the α-carbon by the iron−hydroperoxo species is investigated using a model with full protoporphyrin IX to confirm our previous conclusion that this species does not have sufficient oxidizing power for heme oxidation (*J. Am. Chem. Soc.* **2004**, *126*, 3672). Calculated activation energies and structures of the intermediates and transition state for this process remain unchanged from those for a small model with porphine in the previous study, which shows that the inclusion of the side chain of the porphyrin ring is not essential in describing the OH group transfer. The activation barrier for a direct oxo attack to the α-carbon by an iron−oxo model is calculated to be 49.8 kcal/mol, the barrier height of which looks very high for the enzymatic reaction under physiological conditions. This large activation energy is due to a highly bent porphyrin structure in the transition state. However, a bridging water molecule plays an important role in reducing the porphyrin distortion in the transition state, resulting in a remarkable decrease of the activation barrier to 13.9 kcal/mol. A whole-enzyme model with about 4000 atoms is constructed to elucidate functions of the protein environment in this enzymatic reaction using QM/MM calculations. The key water molecule is fixed in the protein environment to ensure the low-barrier and regioselective heme oxidation. A water-assisted oxo mechanism of heme oxidation by heme oxygenase is proposed from these calculational results.

1. Introduction

Heme oxygenase (HO) catalyzes the conversion of heme to biliverdin, free iron ion, and CO using O_2 and NADPH, as shown in Scheme 1.[1,2] In this process, the heme participates as both prosthetic group and substrate. The porphyrin ring is regioselectively oxidized at the α-position because only the α-*meso*-carbon is accessible to an iron-bound oxidizing species in the protein environment of the active site. A crystal structure of human HO-1 revealed that heme is incorporated into the active center of HO between two helices, and His25 of the proximal helix coordinates to heme as an axial ligand.[3] Steric influence of the distal helix can permit the regioselective oxygenation of the α-*meso*-carbon.

The first step of this catalytic reaction, the conversion of heme to α-*meso*-hydroxyheme, has been investigated in detail.[1,2] A generally accepted mechanism of the HO reaction consists of several steps: (1) binding of heme, (2) reduction of heme to lead to a ferrous species, (3) binding of molecular oxygen to the ferrous iron center, and (4) the second electron transfer to form an active species that is responsible for the α-*meso* hydroxylation of heme. This catalytic cycle is analogous to cytochrome P450's reaction.[4,5] Thus, at first, the active species of the HO reaction was thought to be an iron−oxo complex, so-called compound I, which is a key oxidant for various reactions of heme enzymes, such as cytochrome P450 and horseradish peroxidase. For example, P450s use the high reactivity of the iron−oxo species to oxidize endogenous substrates and a wide range of drugs and xenobiotics under physiological conditions. This hydroxylation mechanism has been established by many experimental[6,7] and theoretical[8−10] methods. In contrast, the iron−hydroperoxo species has been proposed to carry out the regioselective oxidation of heme at the α-*meso* position in the HO reaction. It was reported that HO reacts regioselectively with ethyl hydroperoxide to give α-*meso*-ethoxyheme.[11] This reaction should proceed through the electrophilic addition of the distal oxygen of the iron−

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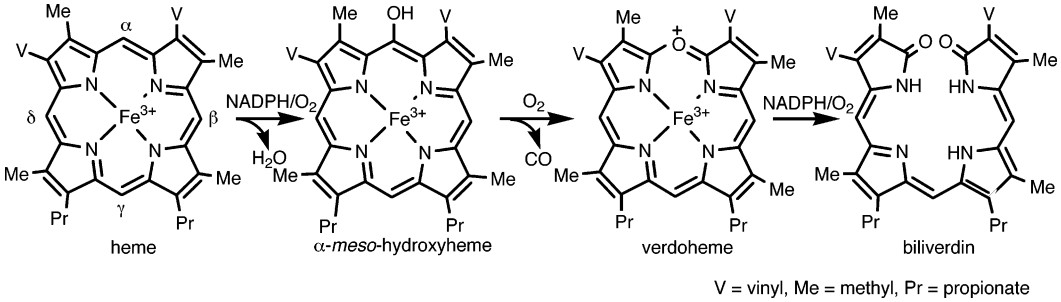
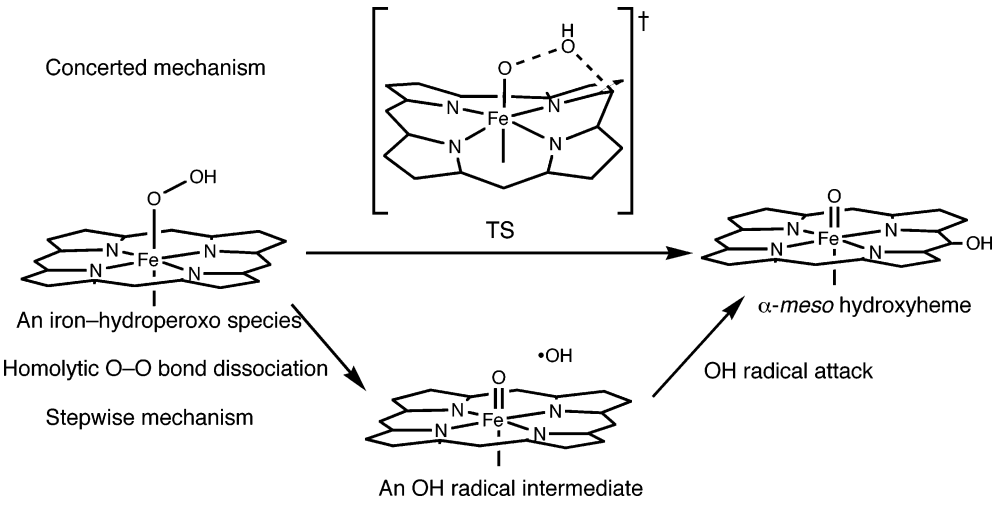
Scheme 1



Scheme 2



hydroperoxo species to the α-carbon. Recent EPR and ENDOR measurements showed that one-electron reduction of an oxy form of the ferrous heme−HO complex by γ-ray at 77 K forms a ferric low-spin species that shows EPR characteristics of an iron−hydroperoxo species, and then its decay leads to the formation of α-*meso*-hydroxyheme.[12]

Density functional theory (DFT) calculations were performed to elucidate the catalytic mechanism of the conversion of heme to α-*meso*-hydroxyheme.[13,14] According to the previous proposals,[11] we considered the energetics for the concerted OH group transfer from the hydroperoxo moiety to the α-carbon, as indicated in Scheme 2. We successfully located the transition state for this step, but the OH group transfer requires a very high activation energy of 42.9 kcal/mol in the doublet state. The poor oxidizing ability of the iron−hydroperoxo species is also suggested for olefin epoxidation by P450.[15,16] DFT calculations indicated that the activation barrier for ethylene epoxidation by the iron−hydroperoxo species is 22.4 kcal/mol, while that by compound I is 9.5 kcal/mol. Unfortunately, the reactivity of this species was found to be comparable to that of hydrogen peroxide itself.[16] Shaik and co-workers[14] also reported a high activation barrier for the concerted attack of the hydroperoxo moiety to the α-*meso*-carbon and proposed a stepwise porphyrin degradation mechanism. This mechanism is initiated by the homolytic cleavage of the O−O bond of the hydroperoxo ligand, followed by the attack of OH radical to the α-*meso*-carbon. The activation energies of the O−O bond dissociation and the OH radical attack were computed to be 20.9 and 0.7 kcal/mol, respectively. This result demonstrates that the stepwise porphyrin oxidation is preferred in energy to the concerted one. However, this stepwise mechanism inevitably involves the formation of the OH radical. It is well-known that the OH radical generated in biological systems is toxic to living things because the radical species damages DNA and cell membranes.[17,18] It is unlikely that this extremely reactive species exclusively reacts with the α-*meso*-carbon rather than with other porphyrin or protein sites.

The purpose of this study is to address the question, "how does heme metabolism occur in heme oxygenase?", which was not fully answered in our previous communication.[13] We reexamine the energetics for the concerted OH group transfer from the hydroperoxo moiety to the α-carbon using an extended model in section 3-1. This calculation supports the previous conclusion that the iron−hydroperoxo species does not have oxidizing ability enough to mediate heme oxidation and thus cannot act as an active species of this reaction. In section 3-2, we propose that a water molecule can act as a key factor of the reaction to reduce the activation barrier for the oxo-mediated pathway. Finally, we present a possible mechanism that will shed new light on the reaction by HO.

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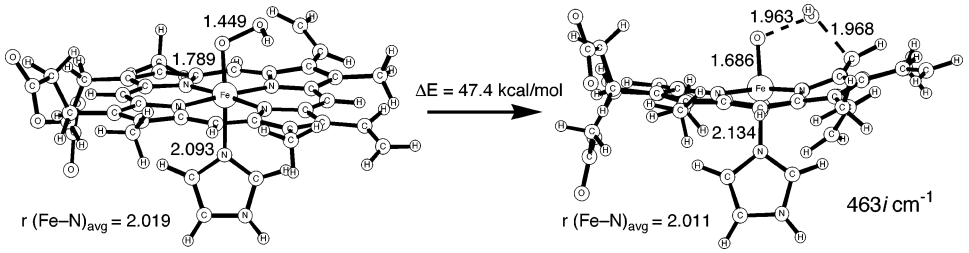

Figure 1. Computed energetical changes in heme oxidation by an iron−hydroperoxo species with protoporphyrin IX in the doublet state. Energy in units of kcal/mol and bond distances in angstroms.

2. Method of Calculation

2-1. QM Calculations. We built iron−oxo and −hydroperoxo species models of HO with imidazole as an axial ligand and a full protoporphyrin IX ring. We calculated the energies and geometries of the reactants, intermediates, and transition states in the doublet and quartet spin states with the B3LYP method,[19] which consists of the Slater exchange, the Hartree−Fock exchange, the exchange functional of Becke,[20] the correlation functional of Lee, Yang, and Parr (LYP),[21] and the correlation functional of Vosko, Wilk, and Nusair.[22] We used the triple-ζ-valence (TZV) basis set[23] for Fe, the D95* basis set[24] for N, O, the α-carbon, and its neighboring atoms, and the D95 basis set for the rest atoms. After full geometry optimizations, single-point calculations were performed, as summarized in the Supporting Information. This result shows that the basis set of choice is sufficient to obtain reliable results. Systematic vibrational analyses characterized stable structures to have no imaginary mode of vibration and transition states to have only one imaginary mode. To test the accuracy of the method of choice in describing the heme oxidation via water-assisted oxo mechanism, we carried out full optimizations of the reactant cluster and the transition state using a recently developed functional called Becke88−Becke95 1-parameter model for kinetics (BB1K), which was reported to be the best density functional-type method to estimate barrier heights.[25] We used the Gaussian 03 ab initio program package for these calculations.[26]

2-2. QM/MM Calculations with a Whole-Enzyme Model. We also built a whole-enzyme model of heme oxygenase based on the crystal structure (PDB ID 1IVJ) of rat hemeoxygenase-1 complexed with heme and azide, as discussed in detail in section 3-3.[27] To make the enzyme model for QM/MM calculations, missing hydrogen atoms were added, and then initial MM minimization was performed while the QM region was fixed. The model with about 4000 atoms was used as an initial structure for the QM/MM calculation with a two-layer ONIOM (IMOMM) method.[28] In these calculations, a specified region around the active site was calculated with the B3LYP method, while the rest of the protein was treated at the Amber96 level of molecular mechanics.[29] The QM region can describe the essential bond-breaking and bond-making processes in the enzyme, while the MM region can promote interactions with the QM region through partial charges and van der Waals forces of atoms in the MM region. At the QM/MM border, atoms in the MM region bound to an atom in the QM region are replaced by hydrogen atoms during the QM level part of the QM/MM calculation. The QM region includes an iron−oxo or −hydroperoxo porphyrin complex (without side chains of heme), the imidazole moiety of His25, the CH_2COO^- moiety of Asp140, the $NHC^+(NH_2)_2$ moiety of Arg136, and important water molecules, as discussed later. The QM/MM calculations require 82 and 81 QM atoms for the iron−oxo and −hydroperoxo species, respectively. We used the TZV basis set for Fe and the D95 basis set for the rest atoms.

3. Results and Discussion

3-1. Concerted Mechanism of Heme Oxidation by the Iron−Hydroperoxo Species. In a previous study,[13] we evaluated the oxygen donation ability of the iron−hydroperoxo species of HO using a small model with porphine in the doublet state. The optimized structure of the iron−hydroperoxo species has O−O and Fe−O bonds of 1.446 and 1.785 Å, respectively. We considered a concerted pathway for the transfer of the distal OH group of the hydroperoxo moiety to the α-carbon. The bond distances of the O−O bond being cleaved and the C−O bond being formed are 2.147 and 1.940 Å, respectively. The activation energy of the transition state for the OH group transfer was calculated to be 42.9 kcal/mol. The effect of zero-point energy correction on the reaction barrier is only 1.3 kcal/mol, the value of which is lower than that for hydrogen atom abstraction processes (2−4 kcal/mol).[30] The essential structural and energetical features are in good agreement with those in a recent study.[14]

To test the validity of the model calculations, in the present study, we considered the side chains of the porphyrin ring using a full protoporphyrin IX heme model. Figure 1 shows optimized geometries of the iron−hydroperoxo species and a transition state for the OH group attack with native protoporphyrin IX. The key structural parameters of the iron−hydroperoxo species are close to those of the small model with porphine; the distances of the O−O, Fe−O, and Fe−N(imidazole) are 1.449, 1.789, and 2.093 Å, respectively. The imaginary frequency mode of 463i cm^{-1} shows that this transition state is responsible for the electronic process of the OH group attack at the *meso* position. The activation energy for this large model was computed to be 47.4 kcal/mol, the value of which is 4.5 kcal/mol higher than that for the previous small model. Thus, effects of the side chains on the OH group transfer process have no impact on the previous conclusion that the iron−hydroperoxo species does not have sufficient oxidizing power for heme oxidation.

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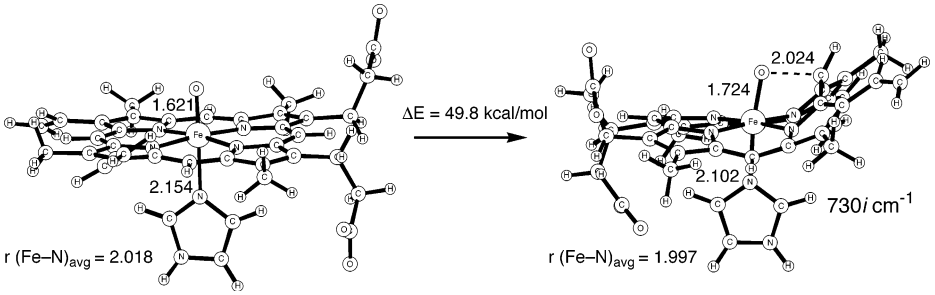

Figure 2. Computed energetical changes in heme oxidation by an iron−oxo species with protoporphyrin IX in the doublet state. Energy in units of kcal/mol and bond distances in angstroms.

3-2. Water-Assisted Oxo Mechanism of Heme Oxidation. Previously, we also considered heme oxidation by the iron−oxo species of HO using a small model with porphine.[13] This reaction is initiated by the creation of a C−O bond between the oxo ligand and the α-*meso*-carbon. The doublet and quartet potential energy surfaces of the reaction are close lying in the initial stages of the reaction. The optimized structure of the transition state has a C−O bond of 2.042 (1.946) Å and an Fe−O bond of 1.697 (1.711) Å in the doublet (quartet) state. The activation energy of the direct attack of the oxo ligand was computed to be 39.9 kcal/mol in the doublet state, the value of which is comparable to that of the iron−hydroperoxo species. The inclusion of ZPE increases the activation barrier by 0.7 (0.5) kcal/mol in the doublet (quartet) state. For these reasons, we tentatively ruled out the oxo-mediated mechanism of heme oxidation by HO. Here, we reconsidered the oxo attack process, taking effects of the side chain of porphyrin into account. Figure 2 shows optimized geometries of the iron−oxo species and the transition state for the oxo ligand attack with native protoporphyrin IX in the doublet state. Unfortunately, the activation energy was calculated again as 49.8 kcal/mol, and thus effects of the side chains of porphyrin are unlikely to be essential for this activation process.

We investigated the mechanism and energetics of ethylene epoxidation by the iron−oxo species of cytochrome P450 with the B3LYP method.[16] The epoxidation reaction takes place in a two-step manner. At first, the oxo ligand of the active species approaches one of the carbon atoms of ethylene to form a C−O bond. The resultant radical intermediate has an unpaired electron located on the terminal CH_2 group. The activation energy of the C−O bond formation is only 9.3 kcal/mol. This process is analogous to the attack of the oxo ligand at the α-position of the porphyrin ring. Thus, we think that the iron−oxo species should have the potential to promote heme oxidation. Why is the barrier for the electronic process so high? The optimized structure of the transition state provided an important clue to this question. We superimposed the porphyrin rings extracted from the optimized structures of the iron−hydroperoxo species and the transition states of heme oxidation by the hydroperoxo and oxo species in Figure 3. This figure clearly shows that the transition state of the oxo attack has a highly bent structure in comparison with that of the hydroperoxo attack. We calculated single-point energies of these porphyrin rings to look at energetical changes coming from the distortion. The porphyrin distortion during the hydroperoxo and oxo attack process was found to bring about the destabilization of the porphyrin ring by 25.1 and 54.5 kcal/mol, respectively. Thus, the geometrical destabilization of the porphyrin ring is likely to prevent the direct oxo attack to the α-*meso*-carbon, despite its high oxidizing power to activate a rigid C−H bond of hydrocarbons.

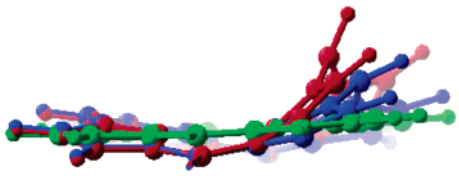

Figure 3. Porphyrin rings extracted from optimized geometries of the iron−hydroperoxo species (green), and the transition states of heme oxidation by hydroperoxo (blue) and oxo (red) species are superimposed.

These results lead us to consider a bridging species that can reduce the structural distortion of the transition state for heme oxidation by the iron−oxo species. To prove this hypothesis, we considered a new oxo-mediated mechanism using a simple model involving an iron−oxo species and a water molecule, as shown in Figure 4. We listed computed atomic charges and spin densities for this process in Table 1. In this new model, the oxo ligand holds a water molecule through a hydrogen bond of 1.950 (1.953) Å in the doublet (quartet) state. This hydrogen-bonding interaction plays an important role in positioning the water molecule to allow the regioselective oxidation of the porphyrin ring. The distance between the oxygen atom of the water molecule and a *meso*-carbon atom is 3.069 (3.103) Å in the doublet (quartet) state. An O−H bond cleavage and a C−O bond formation occur simultaneously in this mechanism. This transition state has only one imaginary frequency mode of 460i (458i) cm^{-1} in the doublet (quartet) state, which corresponds to O−H and C−O stretching motions. The O−H bond of the water molecule and the C−O bond are 1.079 (1.080) and 1.788 (1.786) Å in the doublet (quartet) state, respectively. A calculated activation energy for this step is only 13.9 kcal/mol in both the doublet and quartet states, and it is 26.0 kcal/mol lower than the corresponding value for the direct oxo attack in the previous calculations. The transition state leads to the formation of an intermediate with an intramolecular hydrogen bond between the OH ligand and the migrated OH group. To check the reliability of the B3LYP method in describing this process, we carried out full optimizations of the reactant cluster and the transition state using the BB1K method.[25] We used the TZV basis set for iron and the D95* basis set for all the other atoms. An optimized structure of the transition state, shown in Supporting Information, has an O−H bond of 1.016 Å and a C−O bond of 1.811 Å. An estimated activation energy of 12.6 kcal/mol with this density functional is close to the value obtained at the B3LYP level. The overall reaction is 14.3 and 4.4 kcal/mol exothermic on the doublet and quartet surfaces, respectively, which indicates that the reaction should mainly

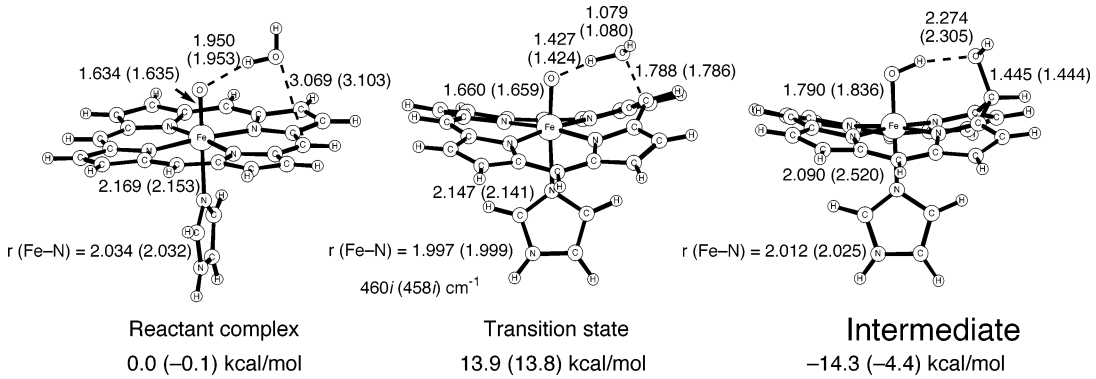

Figure 4. Computed geometrical and energetical changes in heme oxidation by an iron−oxo species with the aid of a water molecule in the doublet (quartet) state. Energy in units of kcal/mol and bond distances in angstroms.

Table 1. Calculated Mulliken Charges and Spin Densities of the Fe and O Atoms and the Imidazole and Porphyrin Moieties (values in parentheses are spin densities) for Heme Oxidation by an Iron−Hydroperoxo Complex with the Aid of a Water Molecule

	Fe	O (oxo)	O (water)	imidazole	porphyrin
			Quartet		
R	1.1 (1.2)	−0.4 (0.9)	0.0 (0.0)	0.2 (0.0)	0.1 (0.9)
TS1	1.1 (1.4)	−0.5 (0.6)	0.3 (0.1)	0.2 (0.0)	−0.1 (0.9)
I	1.3 (2.6)	−0.4 (0.4)	−0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
			Doublet		
R	1.1 (1.2)	−0.4 (0.9)	0.0 (0.0)	0.2 (0.0)	0.1 (−1.1)
TS1	1.1 (1.4)	−0.5 (0.6)	0.3 (−0.1)	0.2 (0.0)	−0.1 (−0.9)
I	1.0 (0.9)	−0.3 (0.2)	−0.1 (0.0)	0.2 (0.0)	0.2 (−0.1)

proceed on the doublet potential energy surface. These results support our idea that a bridging water molecule facilitates the OH group transfer to the α-*meso*-carbon through a less-strained transition state in the oxo mechanism.

3-3. A Possible Mechanism of Heme Oxidation by HO. On the basis of these calculational results, we newly propose an overall mechanism of heme oxidation by HO. The proposed mechanism for the production of the oxo species and heme oxidation is shown in Figure 5. In fact, an iron−hydroperoxo intermediate of HO was captured at 77 K in EPR and ENDOR studies, in which the O_2 moiety of the precursor oxy intermediate is stabilized by a hydrogen bond that would deliver a proton upon electron injection.[12] Mutational studies[31−33] on this enzyme have been extensively performed to investigate the function of key amino acid residues in its active site. Fujii et al.[34] prepared alanine mutants of rat HO-1 (Thr135Ala, Arg136Ala, Asp140Ala, Ser142Ala) and found drastic changes in the enzymatic reactions of Asp140Ala. Stopped flow experiments on Asp140 mutants indicated that the carboxylate at position Asp140 is essential to activate the iron-bound dioxygen and hydroperoxide. They proposed from these results an oxygen activation mechanism involving the hydrogen-bonding network between bridging water molecules and the side chain of Asp140. Noguchi and co-workers[27] determined the crystal structure of rat HO-1 complexed with heme and azide at 1.9 Å resolution. The distance between the azide and the iron atom of heme is 2.2 Å. The azide is nearly parallel to the heme plane and directed toward the α-*meso*-carbon. Raman spectroscopic analyses suggested that the binding mode of azide provides a reasonable model of dioxygen and hydroperoxide binding to heme in HO. The azide ligand interacts with Arg136 and Asp140 via several water molecules in the crystal structure. The hydrogen-bonding network fixes the azide ligand in a proper position for the OH group attack to the α-*meso*-carbon. They proposed that the water molecules also play important roles in the protonation and activation of peroxide bound to heme.

We set up a whole-enzyme model for the iron−hydroperoxo species from the crystal structure only by replacing the azide ligand with a hydroperoxo ligand. Figure 6A shows a QM/MM optimized structure of the iron−hydroperoxo species in this model of HO. Four water molecules (W1−W4) are involved in the QM region to reproduce the essential hydrogen-bonding network in the active site. The QM region we set up does not include Gly143 because it is reported that the hydrogen-bonding and steric effects of Gly143 on the activation energy for heme oxidation are small.[14] The carboxylate anion of Asp140 is hydrogen-bonded to the side chain of Arg136. The distal oxygen of the hydroperoxo ligand is bridged to Arg136 and Asp140 by two water molecules. The O−O bond of the hydroperoxo ligand is slightly longer than that of the small model. This structural change suggests that the protein environment of HO can weaken the O−O bond of the hydroperoxo ligand, which is consistent with the proposal of Noguchi and co-workers[27] that W1 is a potent hydrogen donor to the hydroperoxo ligand. Two water molecules, W1 and W2, are arranged between the COO^- moiety of Asp140 and the hydroperoxo ligand. One hydrogen atom of W1 is hydrogen-bonded to the distal oxygen of the hydroperoxo ligand. These structural features of the hydrogen-bonding network are similar to the proton delivery system of P450 for the conversion of the iron−peroxo species to the iron−oxo species. Mutational studies of P450cam indicated that the Thr252Ala mutation results in a significant decrease of hydroxylation ability,[35] and the replacement of Asp251 with Asn decreases the overall rate of the reaction.[36] A proton inventory analysis of experimental kinetic solvent isotope effect (KSIE) data showed that at least two protons are involved in the formation of the iron−oxo species of P450 and proposed a possible proton delivery model for P450cam involving Asp251, Thr252, and two water molecules.[37] In a previous study, we preformed DFT calculations to elucidate the mechanism of

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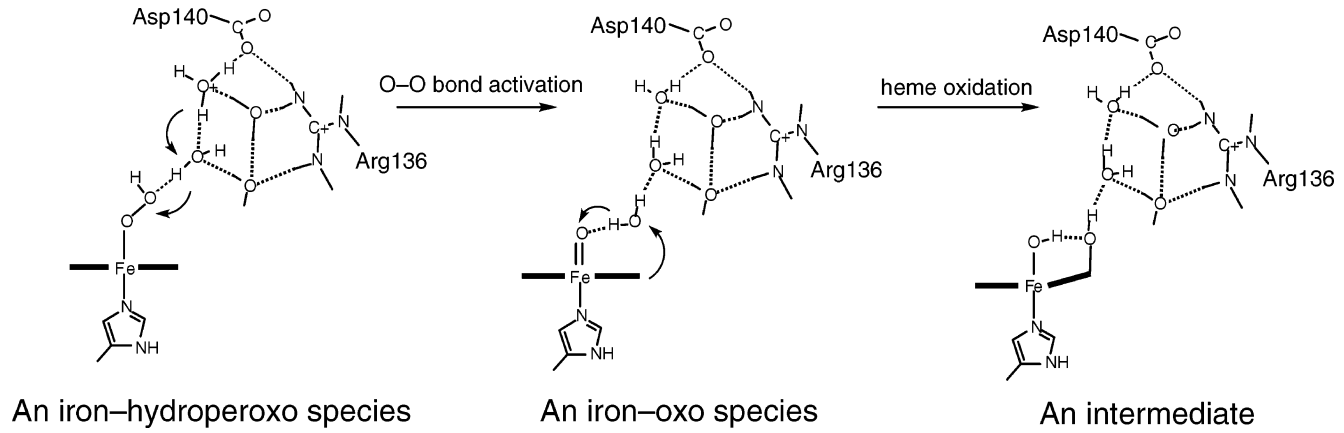

Figure 5. A proposed mechanism of heme oxidation by HO.

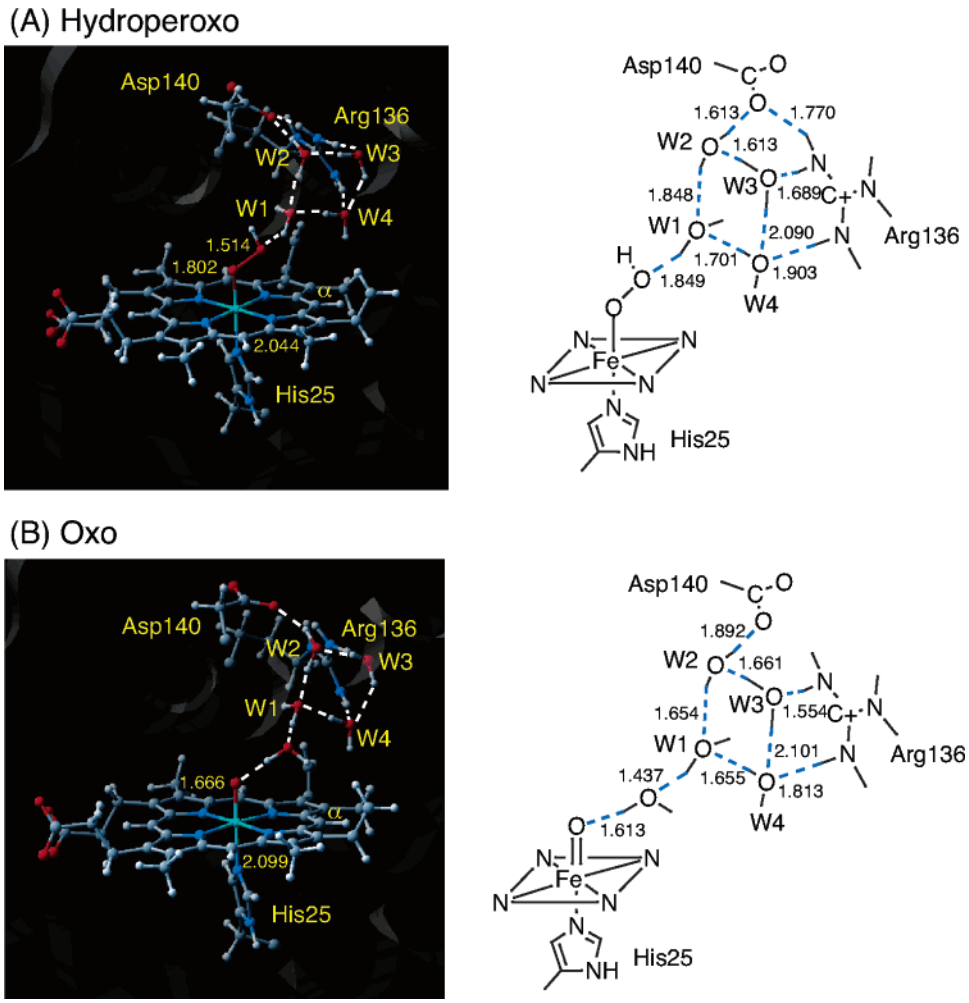

Figure 6. Optimized geometries of an iron−hydroperoxo (A) and −oxo (B) species of HO in the protein environment. Bond distances in angstroms.

the formation of the iron−oxo species of P450cam using a proton source model proposed by them.[9c] The proton transfer process is highly exothermic, and thus, these hydrogen-bond networks can promote effective proton donation to the distal oxygen. Thus, such a proton-transfer mechanism is operative in the active site of HO to give the iron−oxo species, which acts as an active species of heme oxidation.

Figure 6B shows a QM/MM optimized structure of the iron−oxo species of HO in the doublet state.[38] The produced water molecule eliminated from the iron−hydroperoxo species is hydrogen-bonded to the oxo ligand and to W1. The hydrogen bond between the oxo ligand and the water molecule is 1.613 Å in length, and the distance between the oxygen atom of the water molecule and the α-*meso*-carbon is 3.374 Å. The key parameters of the QM/MM optimized structure are close to those of the reactant complex that involves only the iron−oxo complex

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and a water molecule. These structural features enable the effective oxidation of the porphyrin ring with a relatively low barrier using the high oxidation power of the iron−oxo species. The hydrogen-bonding network plays an important role in positioning the bridging water molecule to ensure the regioselective heme activation. The QM/MM optimized structure of the iron−oxo species indicates that steric interactions of the distal helix are also an important factor that fixes the water molecule as proposed.[3] In this mechanism, we assumed that the distal oxygen of the iron−hydroperoxo species is retained in the OH group of α-*meso*-hydroxyheme. Thus, the oxidation of the porphyrin ring would take place without the exchange of the bridging water molecule with solvent. This assumption is reasonable as judged from the rather low barrier of heme oxidation mediated by the iron−oxo species and the interactions that fix the water molecule in the proper position. Ortiz de Montellano and co-workers[11] reported that HO reacts regioselectively with ethyl hydroperoxide to give α-*meso*-ethoxyheme. This result has been considered as strong evidence for the hydroperoxo-mediated mechanism of heme oxidation. However, this experimental result can be rationalized by the present water-assisted oxo mechanism. At first, the O−O bond of the ethylperoxo ligand is heterolytically cleaved by the proton donation from the hydrogen-bonding network to form an iron−oxo species and an ethanol molecule. The ethanol molecule would participate in heme oxidation as a bridging species without the scrambling of the important hydrogen-bonding network. Optimized structures of the reactant cluster and the transition state in this electronic process are shown in Supporting Information. These structures are quite similar to those in the water-assisted pathway; the distance between the oxygen atom of the ethanol molecule and a *meso*-carbon atom is 3.055 Å in the reactant cluster, and the O−H bond and the C−O bond are 1.036 and of 1.792 Å in the transition state, respectively. The activation energy for this reaction was calculated to be only 9.8 kcal/mol. Thus, the distal oxygen of the hydroperoxo and alkylperoxo ligands is highly conserved in the product, which makes it difficult to distinguish between hydroperoxo- and oxo-mediated reactions. A recent mechanistic study on solvent kinetic isotope effects demonstrates that the rate-determining step for the HO reaction must involve not only the C−O bond formation but also the proton delivery to the iron−hydroperoxo species.[39] This is fully consistent with the proposed mechanism.

4. Conclusions

We elucidated the mechanism of heme oxidation mediated by heme oxygenase from DFT computations. At first, we reexamined the energetics for the concerted OH group transfer from the hydroperoxo ligand to the α-carbon, which has been believed to occur in this reaction. Side chain effects of porphyrin were evaluated using an extended model with protoporphyrin IX. The activation barrier for the concerted OH group transfer was calculated to be 47.4 kcal/mol, the value of which is close to that for a small model in which the side chains are replaced by hydrogen atoms. This calculational result strongly supports a previous conclusion that the iron−hydroperoxo species does not have oxidizing power to promote the concerted OH group transfer, and that we have to reconsider the catalytic mechanism of heme oxygenase. We propose a water-assisted oxo mechanism for the function of HO. A bridging species, such as a water molecule, can lower the high activation barrier for heme oxidation by the iron−oxo species because it is helpful in reducing the porphyrin distortion in the transition-state structure. We built a whole-enzyme model with about 4000 atoms to gain a better understanding of the effects of the protein environment on this reaction. The QM region of our QM/MM model involves an iron−hydroperoxo or −oxo complex, Arg136, Asp140, and four water molecules to reproduce the important hydrogen-bonding network between the hydroperoxo ligand and the surrounding amino acid residues. In the QM/MM optimized structure of the iron−hydroperoxo species, Asp140 and two water molecules form a hydrogen-bonding network that connects the distal oxygen of the hydroperoxo ligand. The structural features of the hydrogen-bonding network are similar to the proton delivery system of P450 for the conversion of the iron−peroxo species to the iron−oxo species. The QM/MM optimized structure of the iron−oxo species indicated that the oxo ligand is hydrogen-bonded to the water molecule eliminated from the iron−hydroperoxo species. This water molecule is controlled by the hydrogen-bonding network and steric interactions of distal helix to ensure the low-barrier and regioselective heme oxidation.

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Supporting Information Available: One figure of BB1K optimized structures of a reactant cluster and a transition state for the water-assisted mechanism, one figure of optimized structures for heme oxidation with the aid of an ethanol molecule, complete ref 26, and optimized geometries of all the reaction species. This material is available free of charge via the Internet at http://pubs.acs.org.

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