

Water-Dependent Reaction Pathways: An Essential Factor for the Catalysis in HEPD Enzyme

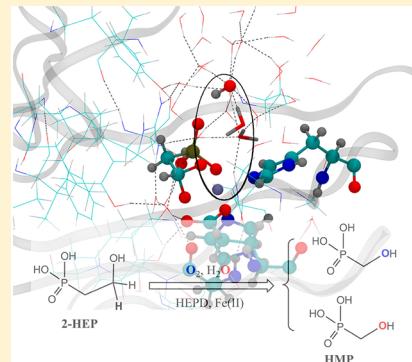
Likai Du,[†] Jun Gao,^{*,†,‡} Yongjun Liu,^{†,‡} and Chengbu Liu^{*,†,‡}

[†]Key Laboratory of Colloid and Interface Chemistry, Ministry of Education, Institute of Theoretical Chemistry, School of Chemistry & Chemical Engineering, Shandong University, Jinan, 250100, P. R. China

[‡]Key Laboratory of Theoretical and Computational Chemistry in Universities of Shandong (Shandong University), Jinan, 250100, P. R. China

Supporting Information

ABSTRACT: The hydroxyethylphosphonate dioxygenase (HEPD) catalyzes the critical carbon–carbon bond cleavage step in the phosphinothricin (PT) biosynthetic pathway. The experimental research suggests that water molecules play an important role in the catalytic reaction process of HEPD. This work proposes a water involved reaction mechanism where water molecules serve as an oxygen source in the generation of mononuclear nonheme iron oxo complexes. These molecules can take part in the catalytic cycle before the carbon–carbon bond cleavage process. The properties of trapped water molecules are also discussed. Meanwhile, water molecules seem to be responsible for converting the reactive hydroxyl radical group ($\cdot\text{OH}$) to the ferric hydroxide (Fe(III)–OH) in a specific way. This converting reaction may prevent the enzyme from damages caused by the hydroxyl radical groups. So, water molecules may serve as biological catalysts just like the work in the heme enzyme P450 StaP. This work could provide a better interpretation on how the intermediates interact with water molecules and a further understanding on the O^{18} label experimental evidence in which only a relatively smaller ratio of oxygen atoms in water molecules (~40%) are incorporated into the final product HMP.



1. INTRODUCTION

The nonheme iron(II) enzymes generally have a 2-His-1-carboxylate facial triad in the active site.^{1–3} The active site of this superfamily of enzymes can be regarded as one of recurring motifs of nature, like heme cofactors and iron–sulfur clusters.⁴ This common structural motif is considered to be a versatile platform for dioxygen activation,^{3,5–7} and it can convert dioxygen to highly specialized reagents and catalyze the synthesis of many important biomolecules in aerobic organisms.^{8,9} Recently, the nonheme iron(II) enzymes have attracted more and more attention, and a number of iron-containing enzymes and related model complexes have been studied by experimental and theoretical works.^{3,10–15}

Hydroxyethylphosphonate dioxygenase (HEPD) is a nonheme enzyme that catalyzes the conversion of 2-hydroxyethylphosphonate (2-HEP) to hydroxymethylphosphonate (HMP).^{16–19} This is a critical step (Figure 1b) in the phosphinothricin (PT) biosynthetic pathway.^{20,21} Cicchillo et al. proposed two reaction mechanisms on the basis of their biochemical experiments and the structure of HEPD, namely, hydroperoxylation and hydroxylation mechanisms.¹⁶ However, these mechanisms cannot provide a comprehensive interpretation for experimental results. Several mechanisms were proposed to explain the experimental results.^{17,22} Recent mutation experimental results show that alternative mechanisms that may involve either phosphite or methylphosphonate as intermediates are not correct.¹⁸ Hence, further studies are

still in need to understand the reaction mechanism of HEPD. Recently, our group has proposed a reaction mechanism based on concatenated bifurcations by employing quantum mechanical/molecular mechanical (QM/MM) and molecular dynamics (MD) simulations.²³ In the proposed mechanism, the carbon–carbon bond cleavage could be achieved through a tridentate binding ligand derived from the hydroperoxo species or employs a proton shuttle-assisted mechanism, in which the concerted proton-transfer process is essential to break the carbon–carbon bond. In the active site, the residue Glu₁₇₆ also contributes to tuning the activity of the enzyme by modulating its protonation state. These results also provided more insight into reaction mechanisms and highlighted the importance of the existence of water molecules.^{16,17,19} The Fe(IV)=O species and a tridentate binding species (Figure 1c,d) were proposed to be important intermediates to break the carbon–carbon bond.

At the same time, our simulation also suggested that water molecules should partially be incorporated into the reaction, which was in agreement with the experimental results.^{16,17} The water-assisted reaction mechanism has been studied in several heme enzymes, such as cytochrome P450 enzymes,^{24–26} hemeoxygenase^{27,28} and KatG enzyme.²⁹ The role of water molecules is believed to provide more additional flexibility in

Received: June 4, 2012

Revised: September 3, 2012

Published: September 5, 2012

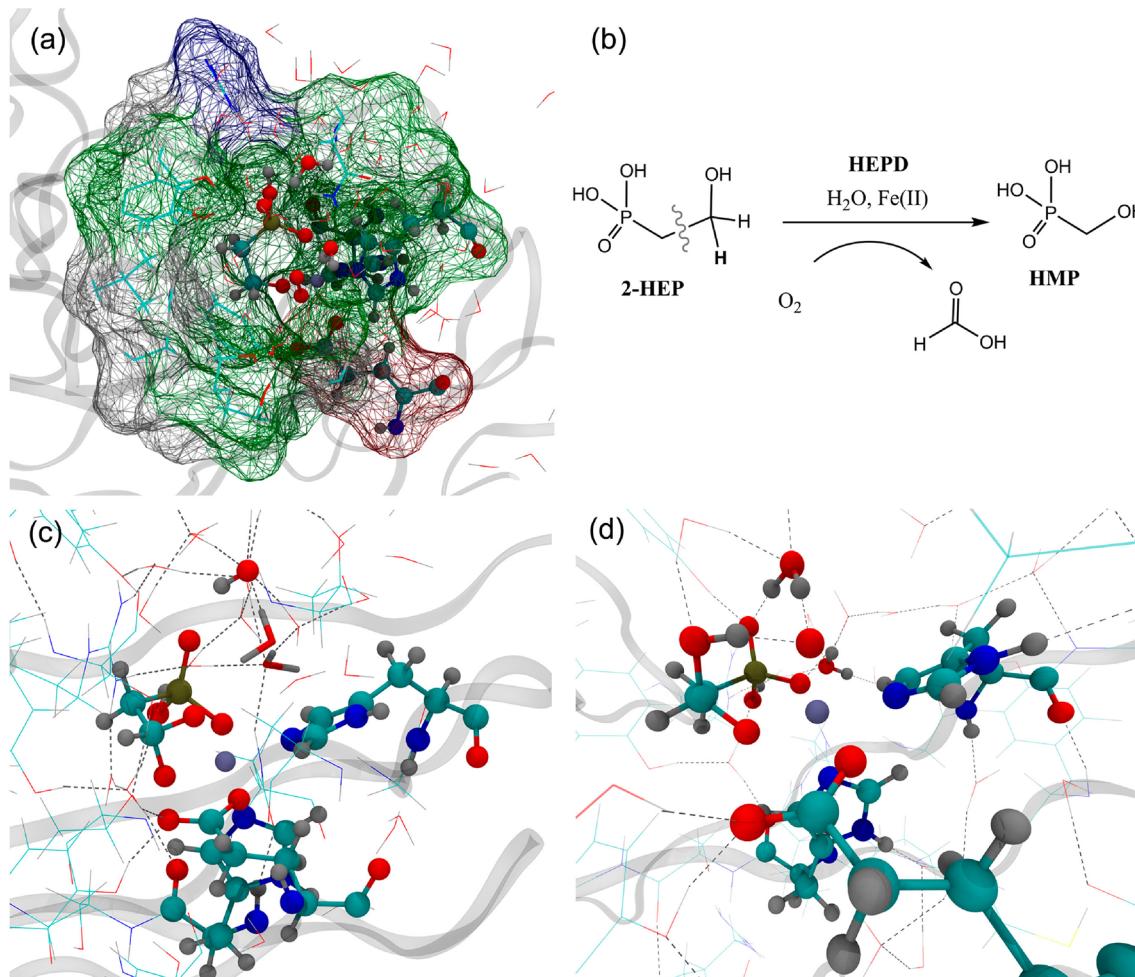
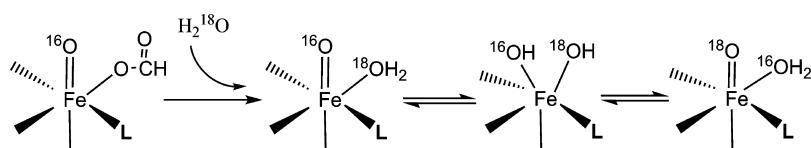


Figure 1. (a) Active-site structures of HEPD (PDB ID 3GBF); the residues about 8 Å from the active center are shown in surface representation. (b) Carbon–carbon bond cleavage in the conversion of 2-HEP to HMP in HEPD. (c) Tridentate binding intermediate. (d) Fe(IV)=O intermediate in the reaction. The hydrogen bonds are shown in gray dashed line; the active center is shown in ball-and-stick model.

Scheme 1. Oxo-Hydroxo Tautomerism Mechanism for ^{18}O Exchange Proposed in Several Non-Heme Iron Models



the multipathway reaction scenario. However, there are few reported experimental and theoretical studies on how the intermediates interact with water molecules in the nonheme enzymes. It is demonstrated in the work of Nam and his colleagues^{30–32} that water molecules are an oxygen source in the generation of nonheme iron model complexes. This is an oxo-hydroxo tautomerism mechanism (Scheme 1). Hirao et al. also suppose that the formic acid produced after the C–C bond cleavage step may be displaced by a water molecule.²² However, because of the steric effects of the surrounding residues (Figure 1a), this reaction was not very easy to take place in our QM/MM calculations. The recent proposed reaction mechanism based on ONIOM calculations by Hirao et al. also considers that the formic acid is retained in the whole reaction pathway.³³

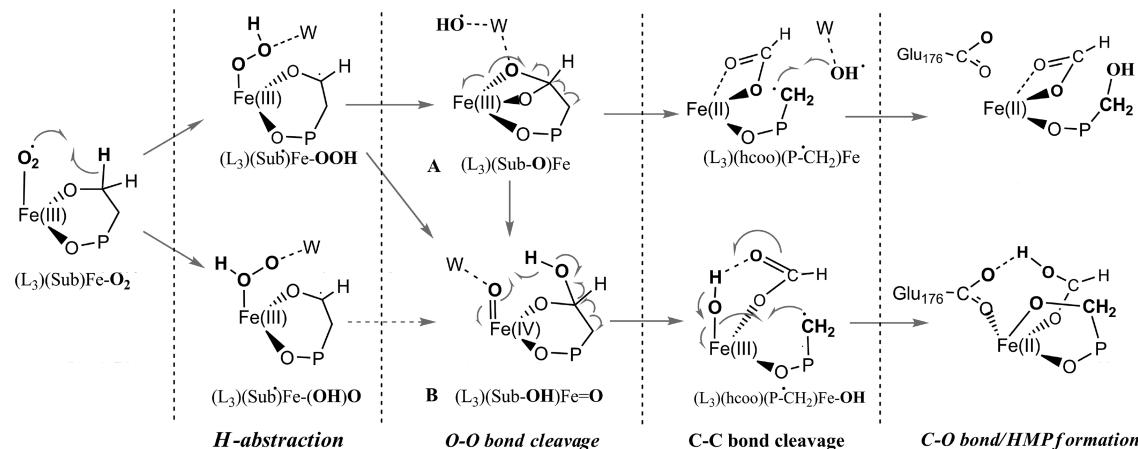
This work attempts to solve two problems: how do the intermediates interact with water molecules, and could it be an

important factor to determine the reaction pathway? To find their answers, we theoretically investigated the water-involved catalytic mechanism and elucidated how water molecules contribute to the whole reaction. This insight into the interplay between intermediates and water may provide a further understanding on the reaction mechanism of HEPD.

2. COMPUTATIONAL DETAILS

The selected model of HEPD in this study was obtained from the QM/MM calculations previously described.²³ The QM/MM procedure in our work was similar to that in our previous studies. A brief summary of important details of the methods is given below.

The QM/MM computations were done by way of ChemShell³⁴ package integrating Turbomole³⁵ and DL-POLY³⁶ programs. The electronic embedding scheme³⁷ was used, and MM charges were incorporated into the one-electron

Scheme 2. Possible Reaction Mechanisms in the Conversion of 2-HEP to HMP in HEPD Enzyme^a

^aKey intermediates are shown. The “Sub” refers to the substrate (2-HEP) in different states; (L_3) is the 2-His-1-Glu motif. Here the key intermediates in the C–C bond cleavage step are abbreviated as Species A and B.

Hamiltonian of the QM calculation. We selected hydrogen as link atoms and adopted the charge shift model³⁷ at the QM/MM boundary region. The final QM/MM model of HEPD contained about 40 000 atoms. The CHARMM22 force field running through the DL-POLY program was used for the treatment of the MM region. The QM region included the 2-His-1-Glu facial triad (His_{129} , His_{182} , Glu_{176}), HEP, O_2 , and two water molecules, and the total charge of the QM region was -1 . The GGA functional PBE was selected to describe the QM region, which has been discussed in the previous work.²³ Singlet, triplet, and quintet states were considered in this study. Two basis sets were employed: B1 [Watchter+ff [$8s6p4d1f$] (Fe)/6-31+G* (the coordinated atoms and the oxygen molecule)/6-31G** (rest)] was used for geometry optimizations, and B2 [Watcherd+ff (Fe)/6-31++G** (rest)] served only for single-point calculations.^{38,39} The details of the classical MD simulations and the QM/MM calculations are given in the Supporting Information. We also performed a 10 ps QM/MM MD simulation to study the iron-containing active center, and the protocols were also given in the Supporting Information.

The main purpose of this study is to elucidate how water molecules take part in the catalytic reaction. Therefore, two water molecules were included in the QM region, and the others were included in the MM region. They were represented as point charges that interacted with the QM region. Because only one or two water molecules could directly take part in the catalytic reaction, such a treatment highlights the role of water molecules as biological catalysts, as in the example of the P450 enzyme.²⁵ However, it might be interesting to extend water cluster sizes when one wants to study the role of water to better understand how the solvent influences the reaction, that is, the cooperative water networks.⁴⁰

3. RESULTS AND DISCUSSION

3.1. Summary of the QM/MM Calculations. The possible mechanisms of the carbon–carbon bond cleavage of 2-HEP have been studied in previous studies.²³ The overall reaction mechanism is presented in Scheme 2. After the dioxygen is bound to the iron center, the dioxygen-bound species ($Fe-O_2$) is generated. This enzymatic reaction occurs in four major steps on the basis of our QM/MM calculations. The first step is the abstraction of hydrogen atom from the

substrate, which leads to a distal or proximal hydroperoxo species ($Fe(III)-OOH$). This is the rate-limiting step, which has an energy barrier of 21 and 18 kcal/mol for distal and proximal H-abstraction processes respectively. The second step is the cleavage of the O–O bond, and the carbon–carbon bond is broken subsequently. In this step, the tridentate binding species (A) and the $Fe(IV)=O$ species (B) are important intermediates to break the carbon–carbon bond. In the third step, the formic acid and the intermediate $^{\bullet}CH_2PO_2(OH)^-$ are generated. Finally, 2-HEP is converted to HMP, and the formate ($HCOO^-$) or formic acid ($HCOOH$) is formed depending on the different reaction pathway in Scheme 2.

Our proposed reaction mechanisms have some differences from the previous proposed ones. The reaction mechanism proposed by Hirao et al. suggests that the $PO_2(OH)^-$ group of the substrate directly takes part in the proton transfer in the reaction cycle.²² In their model, the presence of water molecules was not considered. In our QM/MM model, the $PO_2(OH)^-$ group was surrounded by a few water molecules. So the $PO_2(OH)^-$ group cannot directly take part in the reaction because the water molecules would interfere or prohibit this kind of direct interaction in our QM/MM model. Therefore, it is slightly artificial to interpret this enzymatic reaction without considering the presence of water molecules. We also noted that their recent ONIOM model did not consider the solvation of the protein in water environment.³³ Hence, phosphonate oxygen can also directly interact with the active center in their ONIOM model. Interestingly, in both the ONIOM model of Hirao et al. and our QM/MM model, the phosphonate oxygen of $Fe(III)-OOH$ species was found to easily dissociate from the iron center.

In this work, we mainly focused on the reaction properties of the water molecules. Two key points were selected as presentations. One was the initial state of the reaction, and the properties of water molecules in the active site would be discussed. The other was the second step of the reaction, where the catalytic mechanism of water-promoted reactions (Figure 3) occurred with the O–O bond and the C–C bond cleavage. These two points will be further discussed in the following parts.

3.2. Properties of Trapped Water at the Active Site.

Water molecules play a significant role in governing the biological activity of large folded biomolecules.^{41,42} In our

classical MD simulations, the solvent accessible surface area (SASA) contribution from the hydrophilic residues would increase $\sim 10\%$ after the HEPD enzyme was solvated in water. We also performed the MD simulation based on the QM/MM potential for 10 ps. This provided a better description for the iron-containing active center. It revealed that several water molecules near the active center were immobile during the simulation.²³ Here these water molecules are referred to as “trapped” water molecules. These molecules provide the electrostatic environment for the active site together with the secondary active-site residues in the HEPD enzyme and anchor the substrate 2-HEP in the active site through hydrogen bonds¹⁸ (Figure 1c,d).

As shown in Figure 1a, only a part of the active site is exposed to the water environment (the top side). So, the water molecules can only approach limited region of the active center, around the phosphonate group of the substrate and the O₂ group. At the same time, the limited volume of the active site allows only a few water molecules to interact directly with the active center. The orientations of the trapped water molecules are important for understanding the interplay of water molecules and reactive intermediates. On the basis of the key intermediate A (Figure 1c), various attempts have been made to obtain the possible orientations of the trapped water molecules in the active site (Scheme S1 of the Supporting Information). The results suggest that water molecules are more possible to form a chain than a ring, partially due to the volume limitation of the active site (Figure 1a). These molecules prefer to be located near O₂ and the phosphonate group of the substrate, and the optimized structures are listed in Figure 2.

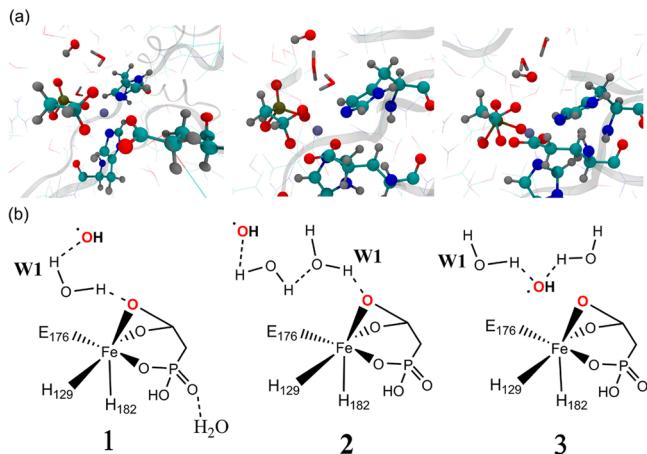


Figure 2. Possible orientations of the trapped water molecules in the active site. (a) Optimized geometries in QM/MM calculations. (b) Schematic representation of the possible orientations. W1 refers to the water molecule that takes part in the reaction; the oxygen atoms derived from O₂ molecule are shown in red color.

Three different spin states were considered. The triplet state is ~ 5 and 11 kcal/mol lower in energy than singlet and quintet states for all structures. The relative energy of the septet state is ~ 20 kcal/mol higher than that of the singlet state, so the septet state is not discussed here. The relative energy of Structures 2 and 3 is almost the same for three spin states, whereas the energy of Structure 1 is 2 kcal/mol higher than that of Structures 2 and 3. The optimized Fe–O_{W1} distances in Structures 1, 2, and 3 are 3.8, 4.0, and 4.5 Å, respectively.

Structures 2 and 3 yield water dimers and have more hydrogen bond interactions (Figure 2). Considering that the increment of Fe–O_{W1} distance may prevent the water dimer from approaching the iron center, only Structure 1 is judged as a suitable initial structure, which has a shortest Fe–O_{W1} distance. In addition, the HOMO–LUMO gap of Structure 1 is only 0.1 eV, whereas the HOMO–LUMO gaps for Structures 2 and 3 are both ~ 0.5 eV, which indicates that Structure 1 is more reactive.

The motion of trapped water molecules in the active site is relatively limited due to the steric effect. The physical and chemical properties of these molecules may be changed by such environments.⁴¹ We use the NPA partial charges to study the polarization of the trapped water molecules. For comparison purpose, three models were used, that is, the single water molecule in the gas phase, the bulk water molecules, and the trapped water molecules in our QM/MM model. A cluster model with 40 water molecules was built to represent the bulk water. The bulk water molecules are strongly polarized in contrast with the gas-phase water molecules. For our QM/MM model, an interesting result is that the water molecule taking part in the reaction (labeled as W1) is only slightly polarized compared with the gas-phase water molecule. So, the reactive water molecule (W1) is very similar to the gas-phase water molecule. Meanwhile, the other water molecule W2 in our QM/MM model is strongly polarized by the environment. The NPA charge of this water molecule (W2) is slightly larger than that of the bulk molecule. The second water molecule W2 prefers to locate near the phosphonate moiety of 2-HEP, and it bridges the interaction between Arg₉₀ and the phosphonate moiety of 2-HEP. At the same time, the site-directed mutagenesis studies of Arg₉₀ and Tyr₉₈ reveal that they are important not only for binding 2-HEP but also for orienting the substrate.¹⁸ So, the relatively larger polarization of W2 may enhance the electrostatic interaction between the Arg₉₀ and the phosphonate moiety.

3.3. Water-Promoted Reaction Mechanism. The water-promoted reaction (Figure 3) occurs along with the O–O bond cleavage and the C–C bond cleavage steps. In the beginning, the Fe–O_w distance is relatively long (~ 3.8 Å) in Species A. The water molecule forms hydrogen bonds with the hydroxyl group and the oxygen atom of the substrate. They are the initial configuration of this reaction. Then, the hydrogen atom in the water molecule is transferred to the oxygen atom of the substrate, whereas the other hydrogen atom in the water molecule migrates to the hydroxyl radical (·OH). In the hydrogen abstraction process, the oxygen atom in the water molecule (W1) replaces the oxygen atom (O_s) in the substrate. After this, Species B is generated, which is a typical Fe(IV)=O species.⁴³ In the following C–C bond cleavage process (Scheme 2), the hydrogen atom in the substrate can easily shuttle back to the Fe(IV)=O motif, and the Fe(III)–OH species would be generated, which could promote the carbon–carbon bond cleavage of the substrate.²³ The relevant optimized structures are also given in Figure S3 of the Supporting Information. This catalytic reaction mechanism (Figure 3) suggests that water molecules serve as biological catalysts, which is similar to the heme enzyme CYT P450 StaP.²⁵ In CYT P450 StaP, the enzyme loses its activity if the dichlorinated substrate (CCA) expels the water molecule.²⁵

In this reaction mechanism, the oxygen atom of the water is partially incorporated into the final product HMP, which is helpful to interpret the experimental evidence.^{16,17} The

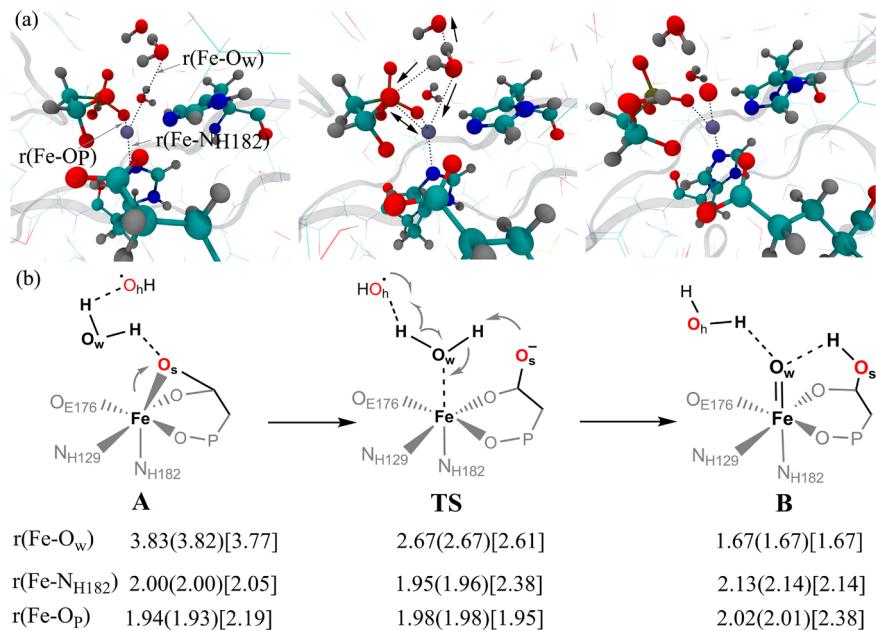


Figure 3. (a) Optimized geometries of key species during the water-molecule-promoted reaction mechanism. The arrows in TS schematically indicate how atoms move in subsequent steps; the active center is shown in ball and stick model. (b) Schematic representation of the reaction. Several key geometric parameters (in angstroms) are given for singlet, triplet (in parentheses), and quintet states (in square bracket); the oxygen atoms derived from O_2 molecule are shown in red color.

hydroxyl oxygen in the HMP product is suggested to derive partially from molecular oxygen and partially from solvent in previous experimental studies.^{16,17} This is an unexpected result because, under the reaction conditions, the primary alcohol of HMP does not exchange oxygen atoms with water. Therefore, they propose the existence of an intermediate species in which oxygen derived from O_2 exchanges with water. Certainly, water as an oxygen source has been reported in a few nonheme model complexes (Scheme 1) by Nam et al.³⁰ As shown in Scheme 1, this oxo-hydroxo tautomerism mechanism indicates that the oxygen atom exchange occurs between the $Fe(IV)=O$ species and the water molecule. In the HEPD enzymatic reaction, the water molecule displaces the formic acid, and the ligand (L) could be the $\cdot CH_2PO_2(OH)^-$ radical. In the mechanism proposed by Hirao et al., the formic acid produced in the C–C bond cleavage step may be displaced by a water molecule.²² In this process, the reaction mechanism is similar to Scheme 1. In their work, the mechanism in which the water oxygen is incorporated into HMP is less favorable, which requires the extra energy barrier of 11.9 kcal/mol, but their proposed mechanism still seems to explain the ^{18}O label experiment.

However, this displacement was not easy to happen in our QM/MM model calculations. In fact, the substrate 2-HEP is surrounded by a few residues (in Figure 1a), and the movement of the substrate is limited. Thus, the displacement of the formic acid with water molecules requires the cooperative motion of these residues, but the $\cdot CH_2PO_2(OH)^-$ radical is an active species, which should react much faster than the oxo-hydroxo tautomerism in such enzymatic environment. Interestingly, the recent proposed reaction mechanism based on the ONIOM model by Hirao et al. also suggested that the formic acid was retained in the formation of HMP.³³ Hence, this oxo-hydroxo tautomerism is considered to be a better choice in the case of a nonheme model complex and may not be easy to happen in this enzymatic environment.

The energy profile of this reaction is given in Figure 4. For the singlet state, the energy barrier is ~ 25.4 kcal/mol, but it is

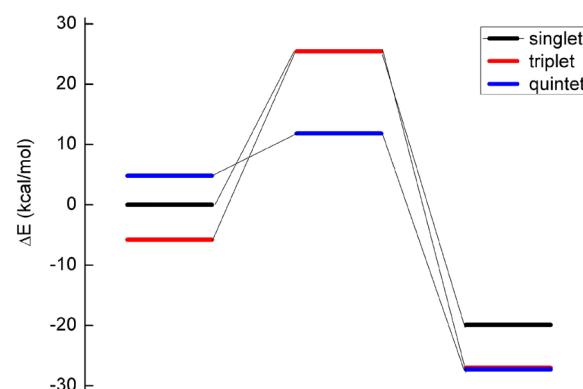


Figure 4. Energy profile of the water promoted reaction mechanism (in kcal/mol). The energy of the singlet state is taken as the reference.

relatively higher (31.2 kcal/mol) in the triplet state. For the quintet state, the energy barrier is much lower (~ 8.0 kcal/mol). The species A in triplet state is the most stable, and it is ~ 10 kcal/mol lower in energy than that of the quintet state. Hence, the scenarios of spin-state crossing are important for this reaction (Figure 4). As shown in Figure 4, there exists a minimum energy crossing section on the reaction pathway. This means that a few spin states of the active species are involved in the reaction mechanism. These systems can exist in several possible spin states, and their order depends on the other ligand bound with iron as well as environmental perturbations.⁴⁴ Therefore, this reaction takes place via multistate reactivity patterns on competing singlet, triplet, and quintet spin-state surfaces, which is similar to dioxygen activation by CDO enzymes.¹ In addition, if the quintet state of the reactant is a metastable state, then this reaction mechanism

will prefer the quintet state. For the quintet state, the transition state (TS) has a long Fe–N_{H182} distance and a slightly shorter Fe–O_p bond. In several heme enzymes, proximal ligands are proposed to tune the reactivity of the iron(IV)–oxo porphyrin π-cation radical intermediates, collectively designated as Compounds I (Cpd I).⁴⁵ This tuning effect of the axial ligand has also been observed in the nonheme complex,^{46–48} such as [Fe(IV)(O)(TMC)(X)]ⁿ⁺. It suggests that the behavior of these complexes in both oxo-transfer and H atom abstraction reactions should systematically depend on the electron-donating ability of the axial ligand in nonheme complexes.^{47,48}

Without the enzymatic environment, the energy barrier is shifted about 4 to 8 kcal/mol higher. Additionally, an alternative reaction pathway is also possible in the singlet state (Figure S5 of the Supporting Information). In such conditions, two hydrogen abstraction processes are stepwise. This stepwise mechanism was not observed in triplet and quintet states. Because the energy barrier is relatively higher (25.6 kcal/mol), we conclude that this stepwise reaction pathway is not favorable.

Here we estimated and validated the relative ratio of oxygen atoms derived from water molecules in HMP. As shown in Scheme 2, the Fe(III)-OOH or Fe(III)-(OH)_O species is generated after the H-abstraction step. Because the Fe(III)-(OH)_O species requires more steps than Fe(III)-OOH species to yield species B, this reaction pathway is less favorable.²³ Therefore, only the Fe(III)-OOH species was considered in our estimation. The Fe(III)-OOH species could convert to species A or species B, and their energy barriers (about 13 and 14 kcal/mol) are very close.²³ So, we supposed that ~50% of Fe(III)-OOH species would convert to species A. For species A, two reaction pathways are possible. However, the energy barrier of the water-promoted reaction pathway is only ~8 kcal/mol, whereas the other one is ~17 kcal/mol. Furthermore, species B is much more stable than species A, as shown in Figure 4. Therefore, most of the species A would convert to species B in the water-promoted reaction pathway. Under such condition, ~50% of oxygen atoms in HMP would contain the oxygen atoms (¹⁸O) derived from water molecules (H₂¹⁸O). This is relatively higher than the experimental value (~40%), but the OH group is also supposed to be able to exchange directly with water molecules, as proposed in our previous studies.²³ The energy barrier of this reaction (H₂¹⁸O + ¹⁶OH ↔ H₂¹⁶O + ¹⁸OH) is very low (<1 kcal/mol), and this reaction is reversible. Therefore, in the equilibrium state, ~50% of hydroxyl (¹⁶OH) in species A would exchange with water molecules (H₂¹⁸O). Then, only 25% of ¹⁶OH is retained for the following water-promoted reaction, and this would result in ~25% of HMP, which contains the oxygen atoms (¹⁸O) derived from water molecules (H₂¹⁸O). It is obvious that there are two competitive reactions, and they define the lower and higher limits of the ratio of oxygen atoms derived from water molecules (25% ≤ ¹⁸O_{HMP}% ≤ 50%), and we could take the average value as an indicator of our conclusion, and that is (25% + 50%)/2 = 37.5%. This is very close to the experimental results (~40%). In general, the 40% of oxygen atoms exchanged in the HMP can be interpreted as two kinds of reactions involved the hydroxyl (OH) group.

A hydroxyl group (OH) exists in the active site of the HEPD enzyme. Its spin value is about 0.1 to 0.3 (singlet to quintet), and the NPA charge is about -0.6e. This suggests that the OH should not be a pure anion group and have radical character. This feature can be directly visualized in Figure 5. For Species

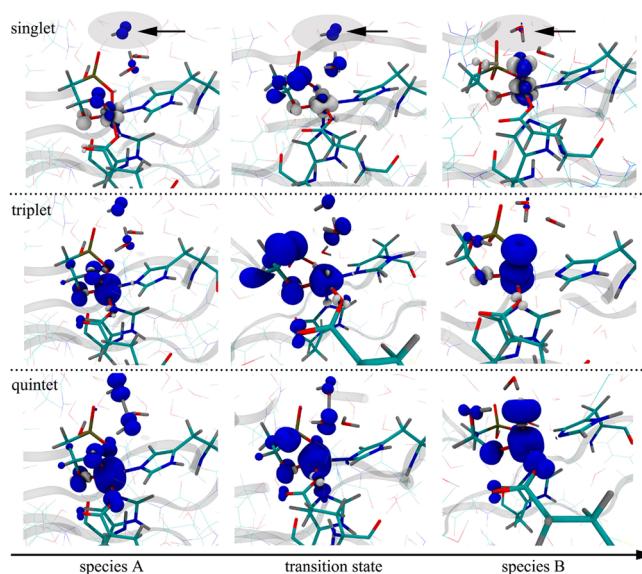


Figure 5. Spin density evolution in the reaction process for (a) singlet, (b) triplet, and (c) quintet states. The spin density gradually disappears in the hydroxyl group; the arrows indicate the position of related hydroxyl group in the reaction. The alpha and beta spin density are shown in blue and white.

A, the spin density is located mainly at the iron atom, hydroxyl group (OH), the substrate, and the water molecule. The spin density on the OH group gradually disappears in the process of this reaction. Finally, in Species B, the spin density is mainly located at the Fe(IV)=O moiety in Species B. This moiety facilitates the carbon–carbon bond cleavage to yield HMP. By removing the electronic environment exerted by the surrounding residues, the spin value of the hydroxyl group increases to 0.6, which indicates strong radical character. To obtain a clearer concept about this radical character of the OH group, the interaction between the hydroxyl group (OH) and W1 in Species A is also analyzed on the basis of a simplified model (water/hydroxyl anion and water/hydroxyl radical). The electron density distribution in the O–H–O plane is given in Figure 6 for anions, radicals, and our QM/MM model. The

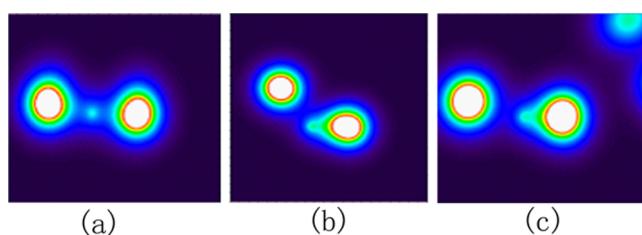


Figure 6. Electron density in the plane of O_{water}, H_{water}, and O_{OH}: (a) water and hydroxyl anion, (b) water and hydroxyl radical, and (c) water and hydroxyl group in the QM/MM model.

electron density distribution in our QM/MM model is similar to that of a water/hydroxyl radical model. So the essence of the interaction between the hydroxyl group (OH) and W1 in the active site may be described as a water/hydroxyl radical model. As a result, the protein environment contributes to eliminate partially the radical character of the hydroxyl (OH). Because the free hydroxyl radicals may cause damages to oxidative cells, DNA, lipids, and proteins, this radical character of OH may be

harmful to the enzyme. So, this reaction mentioned here contributes to the protection of HEPD enzyme.

4. CONCLUSIONS

The trapped water molecule in the active site plays a crucial role in controlling the reaction mechanism. This highlights the importance of the existence of water molecules and provides more insight into reaction mechanisms. These conclusions also give us a clearer understanding about the isotopic labeling experiments. The oxygen atoms derived from water molecules (~40%) in HMP can be the results of two competitive reactions involving the hydroxyl. Furthermore, the water molecule is found in a specific orientation, and it can hold the hydroxyl radical group (OH) tightly. This seems to be responsible for converting a highly reactive hydroxyl radical group to yield a ferric hydroxide (Fe(III)-OH, Scheme 2) in a specific process. The ferric hydroxide is also suggested in previous theoretical and experimental studies.^{18,22} In this reaction, water molecules serve as an oxygen source in the generation of mononuclear nonheme iron oxo complexes.³¹

These results suggest that water molecules should serve as the biological catalyst, which has been proved in the heme enzyme.²⁵ Therefore, the conditions (such as the substrate, mutation) that repel the access of water molecules into the binding pocket may be helpful to inhibit this reaction. Although no other organic cofactor is needed to break the carbon–carbon bond in HEPD enzyme, the water molecule is an important factor in determining the reaction pathway.

■ ASSOCIATED CONTENT

S Supporting Information

Details of the model in the simulations, structure and flexibility of HEPD in water, validation of the ligand/water exchange reactions, and alternative reaction mechanisms and the considered orientations of the trapped water molecules, B-factors per residue of HEPD in water, optimized structures of the QM region, and energy profile of alternative reaction mechanism. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: gaojun@sdu.edu.cn; Phone: +86-531-88363967; Fax: +86-531-88564464 (J.G.). E-mail: cbliu@sdu.edu.cn; Phone: +86-531-88361398; Fax: +86-531-88564464 (C.L.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by the National Natural Science Foundation of China (No. 91127014), Doctoral Fund of Ministry of Education of China (No. 20110131120010), Natural Science Foundation of Shandong Province of China (No. ZR2010BZ005), and Independent Innovation Foundation of Shandong University (2010TS015). It is also supported by Virtual Laboratory for Computational Chemistry, Supercomputing Center of Chinese Academy of Science and Shandong University High Performance Computing Center.

■ REFERENCES

- (1) Hegg, E. L.; Que, L., Jr. *Eur. J. Biochem.* **1997**, *250*, 625–629.
- (2) Neidig, M. L.; Brown, C. D.; Light, K. M.; Fujimori, D. G.; Nolan, E. M.; Price, J. C.; Barr, E. W.; Bollinger, J. M.; Krebs, C.; Walsh, C. T.; et al. *J. Am. Chem. Soc.* **2007**, *129*, 14224–14231.
- (3) Bruijnincx, P. C. A.; van Koten, G.; Gebbink, R. *Chem. Soc. Rev.* **2008**, *37*, 2716–2744.
- (4) Que, L., Jr. *Nat. Struct. Mol. Biol.* **2000**, *7*, 182–184.
- (5) Koehn, K. D.; Emerson, J. P.; Que, L., Jr. *J. Biol. Inorg. Chem.* **2005**, *10*, 87–93.
- (6) Bassan, A.; Borowski, T.; Siegbahn, P. E. M. *Dalton Trans.* **2004**, 3153–3162.
- (7) Friese, S. J.; Kucera, B. E.; Que, L., Jr.; Tolman, W. B. *Inorg. Chem.* **2006**, *45*, 8003–8005.
- (8) Gibson, D. T.; Parales, R. E. *Curr. Opin. Biotechnol.* **2000**, *11*, 236–243.
- (9) Hakemian, A. S.; Rosenzweig, A. C. *Annu. Rev. Biochem.* **2007**, *76*, 223–241.
- (10) Decker, A.; Rohde, J. U.; Klinker, E. J.; Wong, S. D.; Que, L., Jr.; Solomon, E. I. *J. Am. Chem. Soc.* **2007**, *129*, 15983–15996.
- (11) Rohde, J.-U.; Bukowski, M. R.; Que, L., Jr. *Curr. Opin. Chem. Biol.* **2003**, *7*, 674–682.
- (12) Rinaldo, D.; Philipp, D. M.; Lippard, S. J.; Friesner, R. A. *J. Am. Chem. Soc.* **2007**, *129*, 3135–3147.
- (13) Simaan, A. J.; Banse, F.; Girerd, J.-J.; Wieghardt, K.; Bill, E. *Inorg. Chem.* **2001**, *40*, 6538–6540.
- (14) Jensen, K. P.; Bell, C. B.; Clay, M. D.; Solomon, E. I. *J. Am. Chem. Soc.* **2009**, *131*, 12155–12171.
- (15) Decker, A.; Solomon, E. I. *Curr. Opin. Chem. Biol.* **2005**, *9*, 152–163.
- (16) Cicchillo, R. M.; Zhang, H.; Blodgett, J. A. V.; Whitteck, J. T.; Li, G.; Nair, S. K.; van der Donk, W. A.; Metcalf, W. W. *Nature* **2009**, *459*, 871–874.
- (17) Whitteck, J. T.; Cicchillo, R. M.; van der Donk, W. A. *J. Am. Chem. Soc.* **2009**, *131*, 16225–16232.
- (18) Peck, S. C.; Cooke, H. A.; Cicchillo, R. M.; Malova, P.; Hammerschmidt, F.; Nair, S. K.; van der Donk, W. A. *Biochem.* **2011**, *50*, 6598–6605.
- (19) Whitteck, J. T.; Malova, P.; Peck, S. C.; Cicchillo, R. M.; Hammerschmidt, F.; van der Donk, W. A. *J. Am. Chem. Soc.* **2011**, *133*, 4236–4239.
- (20) Schwartz, D.; Berger, S.; Heinzelmann, E.; Muschko, K.; Welzel, K.; Wohlleben, W. *Appl. Environ. Microbiol.* **2004**, *70*, 7093–7102.
- (21) Blodgett, J. A. V.; Thomas, P. M.; Li, G.; Velasquez, J. E.; van der Donk, W. A.; Kelleher, N. L.; Metcalf, W. W. *Nat. Chem. Biol.* **2007**, *3*, 480–485.
- (22) Hirao, H.; Morokuma, K. *J. Am. Chem. Soc.* **2010**, *132*, 17901–17909.
- (23) Du, L.; Gao, J.; Liu, Y.; Zhang, D.; Liu, C. *Org. Biomol. Chem.* **2012**, *10*, 1014–1024.
- (24) Altun, A.; Shaik, S.; Thiel, W. *J. Comput. Chem.* **2006**, *27*, 1324–1337.
- (25) Wang, Y.; Chen, H.; Makino, M.; Shiro, Y.; Nagano, S.; Asamizu, S.; Onaka, H.; Shaik, S. *J. Am. Chem. Soc.* **2009**, *131*, 6748–6762.
- (26) Vidossich, P.; Fiorin, G.; Alfonso-Prieto, M.; Derat, E.; Shaik, S.; Rovira, C. *J. Phys. Chem. B* **2010**, *114*, 5161–5169.
- (27) Lai, W.; Chen, H.; Matsui, T.; Omori, K.; Unno, M.; Ikeda-Saito, M.; Shaik, S. *J. Am. Chem. Soc.* **2010**, *132*, 12960–12970.
- (28) Chen, H.; Moreau, Y.; Derat, E.; Shaik, S. *J. Am. Chem. Soc.* **2008**, *130*, 1953–1965.
- (29) Rangelova, K.; Suarez, J.; Metlitsky, L.; Yu, S.; Brejt, S. Z.; Brejt, S. Z.; Zhao, L.; Schelvis, J. P. M.; Magliozzo, R. S. *Biochemistry* **2008**, *47*, 12583–12592.
- (30) Seo, M. S.; In, J.-H.; Kim, S. O.; Oh, N. Y.; Hong, J.; Kim, J.; Que, L., Jr.; Nam, W. *Angew. Chem.* **2004**, *116*, 2471–2474.
- (31) Lee, Y.-M.; Dhuri, S.; Sawant, S.; Cho, J.; Kubo, M.; Ogura, T.; Fukuzumi, S.; Nam, W. *Angew. Chem., Int. Ed.* **2009**, *48*, 1803–1806.
- (32) Sawant, S. C.; Wu, X.; Cho, J.; Cho, K.-B.; Kim, S. H.; Seo, M. S.; Lee, Y.-M.; Kubo, M.; Ogura, T.; Shaik, S.; et al. *Angew. Chem., Int. Ed.* **2010**, *49*, 8190–8194.

- (33) Hirao, H.; Morokuma, K. *J. Am. Chem. Soc.* **2011**, *133*, 14550–14553.
- (34) Sherwood, P.; de Vries, A. H.; Guest, M. F.; Schreckenbach, G.; Catlow, C. R. A.; French, S. A.; Sokol, A. A.; Bromley, S. T.; Thiel, W.; Turner, A. J.; et al. *J. Mol. Struct.: THEOCHEM* **2003**, *632*, 1–28.
- (35) Ahlrichs, R.; Bär, M.; Häser, M.; Horn, H.; Kölmel, C. *Chem. Phys. Lett.* **1989**, *162*, 165–169.
- (36) Smith, W.; Forester, T. R. *J. Mol. Graphics* **1996**, *14*, 136–141.
- (37) Bakowies, D.; Thiel, W. *J. Phys. Chem. A* **1996**, *100*, 10580–10594.
- (38) Wachters, A. J. H. *J. Chem. Phys.* **1970**, *52*, 1033–1036.
- (39) Chen, H.; Ikeda-Saito, M.; Shaik, S. *J. Am. Chem. Soc.* **2008**, *130*, 14778–14790.
- (40) Bonin, J.; Costentin, C.; Louault, C.; Robert, M.; Savéant, J.-M. *J. Am. Chem. Soc.* **2011**, *133*, 6668–6674.
- (41) Wiggins, P. *PLoS One* **2008**, *3*, e1406.
- (42) Guo, F.; Friedman, J. M. *J. Phys. Chem. B* **2009**, *113*, 16632–16642.
- (43) Solomon, E. I.; Decker, A.; Lehnert, N. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3589–3594.
- (44) Shaik, S.; Chen, H.; Janardanan, D. *Nat. Chem.* **2011**, *3*, 19–27.
- (45) Green, M. T.; Dawson, J. H.; Gray, H. B. *Science* **2004**, *304*, 1653–1656.
- (46) Hirao, H.; Que, L., Jr.; Nam, W.; Shaik, S. *Chem.—Eur. J.* **2008**, *14*, 1740–1756.
- (47) Sastri, C. V.; Park, M. J.; Ohta, T.; Jackson, T. A.; Stubna, A.; Seo, M. S.; Lee, J.; Kim, J.; Kitagawa, T.; Münck, E.; et al. *J. Am. Chem. Soc.* **2005**, *127*, 12494–12495.
- (48) Sastri, C. V.; Lee, J.; Oh, K.; Lee, Y. J.; Lee, J.; Jackson, T. A.; Ray, K.; Hirao, H.; Shin, W.; Halfen, J. A.; et al. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 19181–19186.