J|A|C|S
COMMUNICATIONS

Published on Web 05/02/2007

The Effect of a Water Molecule on the Mechanism of Formation of Compound 0 in Horseradish Peroxidase

Etienne Derat,[†] Sason Shaik,*[,†] Carme Rovira,[‡,§] Pietro Vidossich,[‡] and Mercedes Alfonso-Prieto[‡]

Department of Organic Chemistry and the Lise Meitner-Minerva Center for Computational Quantum Chemistry, Hebrew University of Jerusalem, 91904 Jerusalem, Israel, Centre especial de Recerca en Química Teòrica, Parc Científic de Barcelona, Josep Samitier 1-5, Barcelona, 08028 Spain, and Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys, 23, 08018 Barcelona, Spain

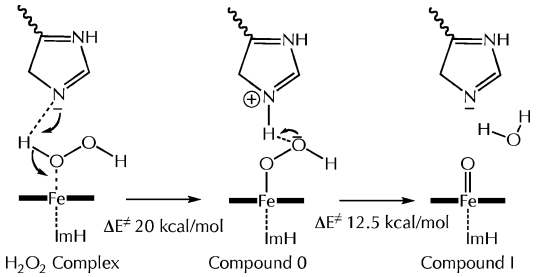
Received October 27, 2006; E-mail: sason@yfaat.ch.huji.ac.il

An important question concerning the catalytic cycles of enzymes is the mechanism of formation of the active species, and the role thereof, of the topology and water content of the active site.[1] Peroxidases are heme enzymes, which utilize hydrogen peroxide (H_2O_2) to form a high-valent iron-oxo species, called Compound I (Cpd I),[1,2] which is the primary active species. Key to the formation of Cpd I are the conserved histidine (His) and arginine (Arg) residues that are strategically placed in the active site and cause proton reshuffle in the iron-coordinated H_2O_2, leading thereby to the heterolysis of the O−O bond and the formation of Cpd I, in the Poulos−Kraut mechanism (Scheme 1). In recent QM/MM calculations,[3] we studied the Poulos−Kraut mechanism of Cpd I formation in horseradish peroxidase (HRP). Surprisingly, the calculated barrier for the first step, deprotonation of H_2O_2 by the conserved His42, leading to Compound 0 (Cpd 0), was quite large, around 20 kcal/mol (Scheme 1). By contrast, kinetics studies on HRP[4] have shown that the barrier for the formation of Cpd 0 is rather small (1.6 ± 0.7 kcal/mol),[4a] and that the rate-limiting step is the O−O bond breaking.[4b] Clearly therefore, the QM/MM computed barrier is in discord with experiment. Already in the original study,[3] we concluded that *the large computed barrier was due to the long distance between His42 and the proximal proton of the Fe−H_2O_2 complex*. But all our attempts to reduce it were not very successful. Something was amiss in this mechanistic jigsaw puzzle! This is the focus of this Communication that addresses the kinetic and acidity of Fe−H_2O_2 and the subsequent bond heterolysis.

Recently, Jones and Dunford have discussed the mechanism of formation of HRP Compound I.[5] According to their analysis, the electrostatic influence of the ferric ion on His42 must be reduced to increase the pK_a of His42 and make it a good enough proton acceptor to deprotonate the hydrogen peroxide. One of the hypotheses along this line was that the active site might contain a water molecule that relays the proton transfer. However, this hypothesis was discarded by analogy to catalase Cpd I formation, which occurs through a "dry" mechanism.[6] Our communication revisits the hypothesis and demonstrates by means of QM/MM calculations that the missing element in the mechanistic jigsaw is precisely *a single water molecule* that enters the active site from the surface of the protein and mediates the deprotonation event at a low barrier and then leaves. As such, we shall demonstrate that unlike catalases, peroxidases function with a "wet" active site.

The QM/MM calculations involved a QM region comprising the heme, H_2O_2, models of Arg38 and His42, and a solvent water molecule, W2407. As shown by the preliminary molecular dynamic (MD) simulations, this water molecule enters the active site quite easily from the surface and is nestled near proline 139 (Pro139) by hydrogen bonds (see SI Figures S1−S4). Following previous studies,[2,3] the QM/MM calculations were done with Chemshell,[7] which interfaces Turbomole for the QM part and the CHARMM force field done by DL_POLY for the MM part. The QM part was carried out with the B3LYP functional and a double-ζ basis set (B1) for energy optimization. Single point energy corrections were performed with a larger basis set (B2). The accuracy of B3LYP was tested with PBE0. The technical details are relegated to the Supporting Information (SI) document.

Scheme 1



The study was limited to the doublet state that was shown to be the ground state surface for Cpd 0.[3] Starting from a configuration in which W2407 is bridging H_2O_2 and His42, the Fe(H_2O_2) complex was optimized by QM/MM and was subject to a follow-up deprotonation. The resulting energy profile is shown in Figure 1 while the critical structures are displayed in Figure 2. As can be seen from Figure 2, the W2407 is engaged in hydrogen bonding with the proximal hydrogen of H_2O_2 and with His42, while one more hydrogen bond is donated by W2407 to Pro139 (present in the MM region; see SI, Figure S4). Thus, the spatial configuration of the QM system is perfectly suited for a proton relay from the hydrogen peroxide through W2407 to His42, resulting in the formation of Cpd 0. Indeed, the transition state (TS_{pt} in Figures 1 and 2) involves a simultaneous double proton transfer: one from the proximal oxygen of H_2O_2 to W2407 and the other from the oxygen of W2407 to the nitrogen of His42. The barrier for this proton relay process is 5.72/5.29 kcal/mol for B3LYP/PBE0, respectively, with the B2 basis set. The barriers in parentheses show the situation in the absence of W2407. *It is apparent therefore, that the single water molecule that relays the proton from the hydrogen peroxide to His42 reduces the barrier for the formation of Cpd 0 by a factor of 3.*[8] The presence of W2407 also stabilizes Cpd 0 (conformation 2 vs conformation 1, Figure 1) significantly. Thus, in the initial conformation, generated directly from TS_{pt}, Cpd 0 is slightly less stable than the Fe−H_2O_2 complex with B2 basis set (conformation 1 in Figures 1 and 2). However, this conformation can be relaxed to a more stable one (conformation 2) that maintains

[†] Hebrew University of Jerusalem.
[‡] Parc Cientific de Barcelona.
[§] Institució Catalana de Recerca i Estudis Avançats (ICREA).

10.1021/ja0676861 CCC: $37.00

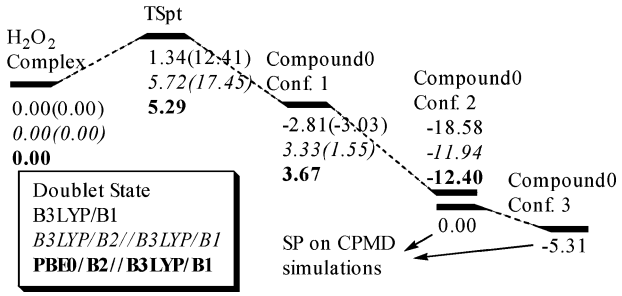


Figure 1. B3LYP/CHARMM energy profile (starting with PDB 1HCH). SP means single point calculations. The data in parenthesis correspond to the values obtained for the same process *in absence of water molecule*.[3]

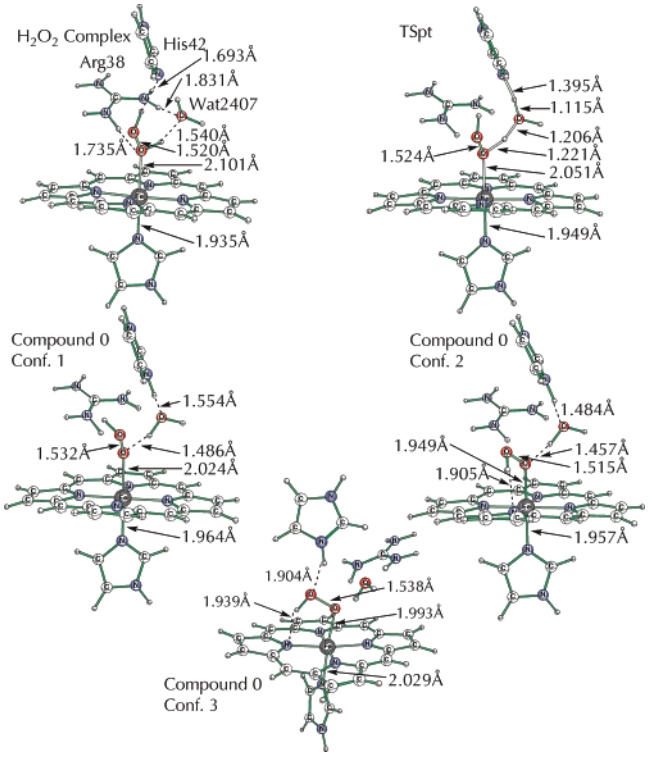


Figure 2. QM/MM optimized structures appearing in Figure 1.

a hydrogen bond between the distal hydrogen and the nitrogen of the porphyrin. In conformation 2, the Fe−O and O−O distances are also shorter compared with conformation 1 (1.949 Å vs 2.024 Å; 1.515 Å vs 1.532 Å), and it is more stable by ca. 15 kcal/mol. Thus, a single water molecule in the active site is seen to also enhance the thermodynamic acidity of H_2O_2 by many orders of magnitude ($pK_a = 11$ for free H_2O_2). The stabilization of conformation 2 can be accounted by two main factors: (i) a new hydrogen bond of the distal proton of the OOH moiety and (ii) Arg38 interacts more strongly with the distal oxygen in conformation 2 (see SI, Table S7). The hydrogen bond between Arg38 and the proximal oxygen reduces the radical character of the OO moiety, which itself shortens both the O−O and the Fe−O bonds thus stabilizing conformation 2.

Interestingly, all our attempts to generate Cpd I either from conformation 1 or from conformation 2 failed. Previously,[3] in the absence of W2407, the protonated His-H^+42 could easily transfer a proton back to the distal oxygen of Cpd 0 and lead to the formation of Cpd I with a barrier of 12.5 kcal/mol (Scheme 1). Now, however, W2407 is in the way; it prevents the formation of a reactive conformation of Cpd 0, like the one we found previously,[3] and it keeps the His-H^+42 far enough from the distal oxygen, thereby preventing its protonation and formation of Cpd I.

To find the mechanism whereby W2407 gets out of the way, we performed CPMD and classical MD simulations (see SI, pp S32−S36) at room temperature. The CPMD simulations show that His-H^+42 exchanges hydrogen bond (H-bond) partners easily, switching back and forth between conformation 2 and conformation 3, in less than 1 ps (Figure S6). As shown in conformation 3 in Figure 2, W2407 moves away and reattaches to the H-bonding network that connects to Pro139 (SI, Figure S7), while at the same time, His-H^+42 makes a H-bond with the distal oxygen of the FeOOH group of Cpd 0. The QM/MM relative energies show that the conformation 3 is slightly more stable (by 5.31 kcal/mol) than conformation 2 (see Figure 1, SI Figure S8). Furthermore, the classical MD simulations show that after 1.3 ns, W2407 departs completely (Figure S9−S10). In conformation 3, and certainly after departure of W2407, Cpd 0 is perfectly set up for the heterolytic bond cleavage precisely as we found before in the Poulos−Kraut mechanism.[3] Thus, rearrangement of W2407 and of the H-bond network in the active site must attend the formation of Cpd I, in accord with the experimental findings of large entropic effects after formation of Cpd 0.[4a] Recent results for P450 further reinforce the crucial role of water molecules in O−O activation.[9]

In summary, we found that a single water molecule has a profound effect on the thermodynamic and kinetic acidities of H_2O_2 without hampering the follow-up formation of Cpd I. Thus, H_2O enters, performs its catalytic action and leaves to make way for an efficient heterolytic O−O cleavage in its absence. This catalytic effect reflects the ease of partner switching in H-bonding and highlights the importance of H-bonding networks in enzymes. The proposed decisive role of a single water molecule, which is connected to Pro139, may be tested by mutating the latter residue.

Acknowledgment. S.S. is supported of the Israel Science Foundation. C.R. is supported by the Spanish Ministry of Science and Education and the Barcelona Supercomputing Center. E.D. thanks the CRIHAN for computing facilities (Project 2006013).

Supporting Information Available: Complete ref 7b; methodology, energies, spin densities, charges and xyz coordinates for all species. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Poulos, T. L. Peroxidases and Cytochrome P450. In *The Porphyrin Handbook;* Kadish, K. M., Smith, K. M., Guilard, R., Eds.; 2000; Vol. 4, Chapter 32, pp 189−218. (b) Dunford, H. B. *Heme Peroxidases;* Wiley-VCH: New York, 1999. (c) Gajhede, M. In *The Handbook of Metalloproteins*; Messerschmidt, A., Huber, R., Poulos, T., Wieghardt, K., Eds.; Wiley: New York, 2001; Vol. 1, pp 195−210.
(2) Derat, E.; Cohen, S.; Shaik, S.; Altun, A.; Thiel, W. *J. Am. Chem. Soc.* **2005**, *127*, 13611−13621.
(3) Derat, E.; Shaik, S. *J. Phys. Chem. B* **2006**, *110*, 10526−10533.
(4) (a) Baek, H. K.; Van Wart, H. E. *J. Am. Chem. Soc.* **1992**, *114*, 718−725. (b) Rodriguez-Lopez, J. N.; Gilabert, M. A.; Tudela, J.; Thorneley, R. N. F.; Garcia-Canovas, F. *Biochemistry* **2000**, *39*, 13201−13209.
(5) (a) Jones, P.; Dunford B. *J. Inorg. Biochem.* **2005**, *99*, 2292−2298. (b) Jones, P. *J. Biol. Chem.* **2001**, *276*, 13791.
(6) Fita, I.; Rossmann, M. G. *J. Mol. Biol.* **1985**, *185*, 21.
(7) (a) ChemShell, a Computational Chemistry Shell. www.chemshell.org. (b) Sherwood P.; et al. *THEOCHEM* **2003**, *632*, 1−28.
(8) A low barrier was reported recently without either water 2407 or the protein environment; see: Zazza, C.; Aschi, M.; Palma, A. *Chem. Phys. Lett.* **2006**, *428*, 152−156. But the QM only study starts from a conformation that brings His42 very close to the proximal proton of Fe(H_2O_2). We note that His42 is in an α-helix that cannot be altered easily to this conformation. Our own QM/MM CPMD simulations do not reveal such a conformation.
(9) (a) Harris, D. L. *J. Inorg. Biochem.* **2002**, *91*, 568. (b) Zheng, J.; Wang, D.; Thiel, W.; Shaik, S. *J. Am. Chem. Soc.* **2006**, *128*, 13204−13215.

JA0676861