How Heme Metabolism Occurs in Heme Oxygenase: Computational Study of Oxygen-Donation Ability of the Oxo and Hydroperoxo Species

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Heme oxygenase (HO) is distributed in a wide variety of organisms such as bacteria, plants, and mammal. A major function of this enzyme is iron homeostasis, freeing iron from heme for reuse.[1] A porphyrin ring is regioselectively oxidized at the α-position to produce biliverdin, carbon monoxide, and free iron using O_2 and NADPH in these reactions, as shown in Scheme 1.[2,3] This enzyme by itself has no porphyrin ligand unlike cytochrome P450, horseradish peroxidase, myoglobin, and hemoglobin. Poulos et al. revealed from the crystal structure of human HO-1 that heme is incorporated into the active center of HO between two helices and proposed that His25 of the proximal helix coordinates to heme as an axial ligand and steric influence of the distal helix permits the regioselective oxygenation at the α-*meso* carbon.[4]

The first step of this catalytic reaction, the conversion of heme to α-*meso*-hydroxyheme, which we focus on in this work, has been extensively investigated. At first, the active oxidant for this reaction is thought to be an iron−oxo complex, so-called compound I, as seen in P450-catalyzed reactions.[5,6] However, Ortiz de Montellano et al. reported that the reaction of HO with ethyl hydroperoxide gives α-*meso*-ethoxyheme and concluded that this reaction proceeds through the electrophilic addition of the distal oxygen of an iron−hydroperoxo species.[7] Recently, Hoffman et al. supported this proposal from EPR and ENDOR measurements.[8]

Shaik et al. and our group estimated the oxygenation ability of the iron−hydroperoxo species of cytochrome P450 from B3LYP density functional theory calculations.[9,10] The activation barrier for ethylene epoxidation by the iron−hydroperoxo species was found to be 12.9 kcal/mol higher than that of 9.5 kcal/mol by compound I. The reactivity of this species is comparable to that of hydrogen peroxide itself.[10] This result leads us to reconsider the hydroperoxo-mediated mechanism. The aim of this communication is to raise questions about the prevailing catalytic mechanism of HO from a theoretical viewpoint.

To increase our understanding of the HO reaction, we performed B3LYP calculations with the Gaussian 98 program.[11] Imidazole and porphine were used as models of an axial ligand and protoporphyrin IX, respectively. We used the 6-31G* basis set for Fe, O, N, α-carbon, and its neighboring atoms and used the 6-31G basis set for the rest atoms.[12] The reliability of this choice for the system is shown in Supporting Information. We confirmed from vibrational analyses that each transition state has only one imaginary frequency mode and correctly connects a reactant and a product.

Figure 1 shows a computed potential-energy profile for the conversion of heme to α-*meso*-hydroxyheme by an iron−hydroperoxo complex. Calculated atomic charges and spin densities for these reaction species are listed in Supporting Information. We considered only the low-spin doublet state because previous
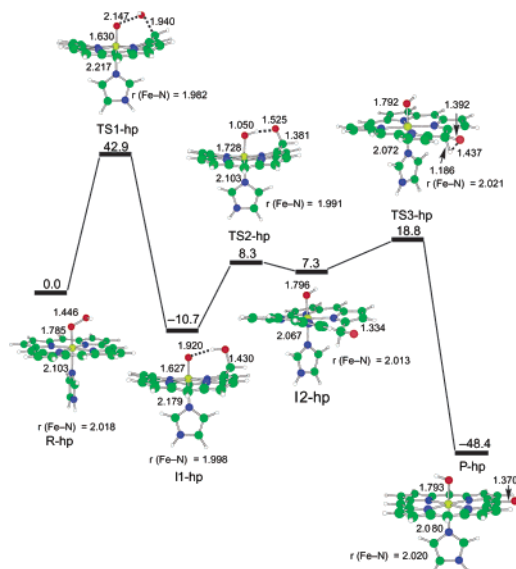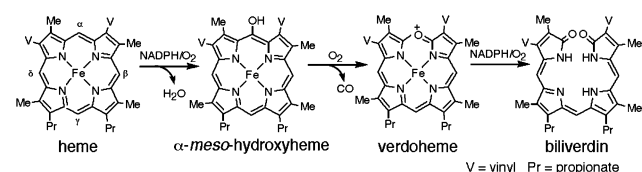
Figure 1. Energy profile (in kcal/mol) for the conversion of heme to α-*meso*-hydroxyheme by the iron−hydroperoxo species of HO. Optimized parameters are also shown in Å.

Scheme 1

heme → α-*meso*-hydroxyheme → verdoheme → biliverdin
V = vinyl Pr = propionate

theoretical[13] and experimental[8] analyses of the iron−hydroperoxo species showed the ground state of this complex to be doublet. The optimized structure of the iron−hydroperoxo species (**R-hp**) has an O−O bond of 1.446 Å. The O−O bond is 0.094 Å shorter than that of CH_3S-coordinating iron−hydroperoxo species, a model of cystein-coordinating cytochrome P450.[10] The change of the O−O bond distance is relevant to the electron-donation ability of the axial ligand. Dawson et al. pointed out a strong electron donation from the negatively charged ligand of cytochrome P450 to facilitate the O−O bond cleavage.[14] In HO, the cleavage of the O−O bond is decelerated, and the transient iron−hydroperoxo intermediate with an extended lifetime can participate in the heme oxidation easier.

This reaction pathway is initiated by the direct attack of the distal OH group of the hydroperoxo moiety to the α-carbon. The optimized structure of the transition state (**TS1-hp**) has an O−O bond being cleaved of 2.147 Å and a C−O bond being formed of 1.940 Å. The spin density of −0.5 on the distal oxygen indicates that the O−O bond can be homolytically cleaved as seen in ethylene epoxidation by the iron−hydroperoxo species of P450.[9,10] The O−O bond cleavage, which is the rate-determining step in this pathway, requires an activation energy of 42.9 kcal/mol. The resultant
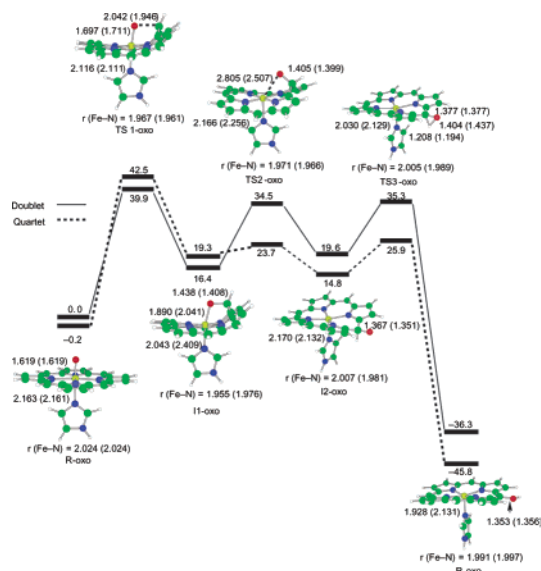
Figure 2. Energy profiles (in kcal/mol) for the conversion of heme to α-*meso*-hydroxyheme by the iron−oxo species of HO in the doublet (quartet) state. Optimized parameters are also shown in Å.

intermediate (**I1-hp**) involves an intramolecular hydrogen bond between the produced oxo oxygen and the migrated OH group. The oxo group of **I1-hp** abstracts the H atom from the OH group via **TS2-hp** to yield **I2-hp** that has an oxygen atom connected to the α-carbon of the porphyrin ring. The spin density of the oxygen was calculated to be 0.5, and the radical character of the oxygen allows the H-atom abstraction from the α-carbon via a three-centered transition state (**TS3-hp**) to form the product complex (**P-hp**), which is a highly exothermic process.

We also considered heme oxidation by the iron−oxo species, which is a main oxidant of P450. The doublet and quartet potential energy surfaces of the reaction are closely lying in the course of the reaction, as depicted in Figure 2. This mechanism is initiated by a porphyrin distortion via **TS1-oxo** to form an unstable intermediate with a highly bent structure, **I1-oxo**. The activation energy of the direct attack of the oxo group to the α-carbon is 39.9 kcal/mol; this high barrier is mainly due to the bent form of the porphyrin ring. To release the structural distortion of **I1-oxo**, the Fe−O bond is cleaved via **TS2-oxo** to produce **I2-oxo**. **I2-oxo** has a spin density of −0.9 and 0.8 on the oxygen atom in the doublet and quartet states, respectively. This oxygen abstracts an H atom via **TS3-oxo** in a way similar to **TS3-hp** to give **P-oxo**.

These energy profiles clearly show that heme oxidation by the iron−hydroperoxo and −oxo species of HO has unusually high barriers of 42.9 and 39.9 kcal/mol, respectively, even if environmental factors slightly change the reactivity of these species. Shaik, for example, showed that the activation energy for the hydroxylation of methane that has very rigid C−H bonds is about 27 kcal/mol.[15] It is difficult to imagine that such reactions with activation energies of 40 kcal/mol proceed under physiological conditions. Although the poor oxidizing ability of the iron−hydroperoxo species is consistent with Shaik's and our previous studies,[9,10] the unexpected

result that the reactivity of the iron−oxo species is comparable to that of the iron−hydroperoxo species is embarrassing in that we must rule out the generally accepted candidates for the active species of the HO reaction. This implies that an overlooked factor without P450 drastically reduces the high barrier or that different unstable intermediates such as an iron−peroxo species act as the true active species. Now we are undertaking further calculations toward a better understanding of the key factor and the true active species that would be the essence of the HO catalytic mechanism.

Supporting Information Available: (a) Two tables of charge and spin densities of all species, and one table of calculated heats of reaction. (b) One figure of imaginary modes of transition states (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA030393C