P450 Sulfoxidation

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Which Oxidant Is Really Responsible for Sulfur Oxidation by Cytochrome P450?**

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Cytochromes P450[1] are universal oxidants that pose tantalizing challenges to mechanistic chemistry primarily because the reactive species of the enzyme is still unidentified.[2–4] The widely accepted reactive oxidant is the iron–oxo porphyrin species Compound I (Cpd I) shown in Figure 1.[4–9] However, a contender oxidant is the precursor ferric hydroperoxide species known as Compound 0 (Cpd 0).[4,6,10–12] The sulfoxidation mechanism of thioethers by P450 highlights this conundrum. Recently, Cryle and De Voss reported results of fatty acid and thiafatty acid oxidation by $P450_{BM3}$ and its T268A mutant, in which it is thought that the cycle stops at Cpd 0, and/or Cpd I formation slows down.[13] Since the features of the fatty acid hydroxylation were affected by the mutation, while those of sulfur oxidation in thiafatty acids remained unchanged, and since the enantioselectivities of the two reactions were opposite, the authors were obliged to conclude that Cpd I performs the hydroxylation, while presumably Cpd 0 is responsible for sulfoxidation. A similar conclusion, albeit less compelling, had been reached[14] for the oxidation of *N*,*N*-dimethyl-4-(methylthio)aniline promoted by the T268A mutant of $P450_{BM3}$.

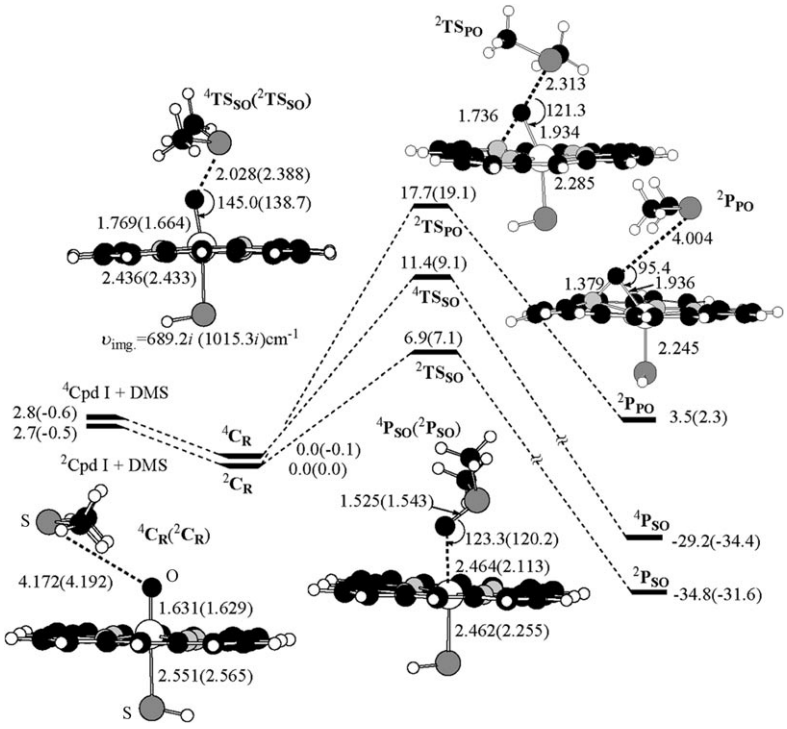

Figure 1. Geometries of key species (B3LYP/LACVP**, bond lengths in Å, angles in °) and energy profiles for the sulfoxidation of DMS by Cpd I. The relative energies (in kcal mol^{-1}) of the species correspond to E_1 (E_2 in parentheses). The values of the imaginary frequencies in cm^{-1} are shown underneath the $^{4,2}\mathbf{TS_O}$ structures.

What makes Cpd 0 a potent oxidant for sulfur, and why does Cpd I not perform this reaction? After all, Cpd I is present in the native enzyme and presumably also in the mutant. The demonstration that Cpd I reagents easily promote sulfoxidation,[15] and that in the nonheme system[16] Cpd 0 is nonreactive compared with the Cpd I species, further exacerbates the dilemma. Which of the two oxidants, Cpd I or Cpd 0, of P450 is intrinsically the more potent oxidant for sulfoxidation? This is the primary question to be answered before this conundrum can be further discussed.

To answer this question, we used theory to examine the sulfoxidation of dimethyl sulfide (DMS) by Cpd 0 by the O–O homolysis mechanism,[17–19] which is exceedingly more facile than the direct O-transfer mechanism.[20] To gauge the relative reactivities of the two oxidants, we examined a few potential paths of sulfoxidation by Cpd I and Cpd 0. The calculations were done on model species with SH^- as the sixth ligand.[4]

Figure 1 displays the results for sulfoxidation by Cpd I in its lowest doublet and quartet spin states. The observed mechanism is seen to involve an O-transfer step via transition states $^{4,2}\mathbf{TS_{SO}}$ that lead to the product complexes $^{4,2}\mathbf{P_{SO}}$. Transition state $^2\mathbf{TS_{SO}}$ is 2.0 kcal mol^{-1} lower (E_2) than $^4\mathbf{TS_{SO}}$ (see also the Supporting Information). The ^{2}**TS** species reported in our former study,[20] here $^2\mathbf{TS_{PO}}$, is found to lead to the porphyrin self-oxidation (PO) product $^2\mathbf{P_{PO}}$.[7] Thus,

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Cpd I will carry out a fast sulfoxidation of DMS with a barrier of 7.1 kcal mol^{-1}.

The O–O homolysis process (Figure 2a) leads to two nearly degenerate intermediates, $^{4,2}\mathbf{I_{O-O}}$, but thereafter (Figure 2b), the quartet-state barriers for the sulfoxidation steps are much higher than the doublet barriers. The sulfoxidation step on the doublet surface in Figure 2b can proceed either by proximal oxygen (O_p) attack, or by attack from the distal oxygen atom of the O_dH radical. The O_d attack, which involves coupled transfers of hydrogen/oxygen atoms, is more facile than the O_p attack. The rate-controlling step of the process is the O–O bond homolysis, with a barrier of 17.8 kcal mol^{-1} (E_2). The inclusion of a water molecule (W) hydrogen-bonded to the FeOOH moiety raises this barrier (E_{2W}). The presence of DMS during homolysis also raises slightly the homolysis barrier and lowers the barrier for O_d attack (Figure S7 in the Supporting Information). Other attempts showed even higher barriers for Cpd 0 reactivity (see the Supporting Information, Part 6). With a barrier of at least 17.8 kcal mol^{-1}, Cpd 0 is an inferior oxidant compared with Cpd I, for which the barrier is 7.1 kcal mol^{-1} (Figure 1).

To define the highest reactivity of Cpd 0 under extreme acidic conditions, we investigated the homolysis/O_d-attack mechanism catalyzed by a $H_2O{\cdot}H_3O^+$ cluster. During the optimization of the Cpd 0/$H_2O{\cdot}H_3O^+$ cluster there occurred a spontaneous Grotthuss-type protonation of the O_p position, leading to an iron hydrogen peroxide complex, $[Fe^{III}(H_2O_2)]^+$.[18,21] The latter complex itself can be another second oxidant or a variant of Cpd 0 involving acid catalysis.[17] The reaction profile (Figure 3) from Fe(H_2O_2) exhibits a stepwise homolytic feature. The barrier for bond homolysis is lowered by the protonation to 15.0 kcal mol^{-1}, followed by a small sulfoxidation barrier of 1.2 kcal mol^{-1}. While the prospects for sulfoxidation through this mechanism are better, the rate-controlling barrier is still 8.3 kcal mol^{-1} higher than with Cpd I. Thus, even under these extreme conditions, which are unrealistic for the enzyme, Cpd I is still the dominant oxidant.

Other considerations for judging the competency of Cpd 0 and Fe(H_2O_2) as oxidants in the presence of Cpd I are the side reactions and the barrier separating Cpd 0 and Cpd I. Thus, after the O–O homolysis of Cpd 0 (Figure 2), the sulfoxidation by $O_dH^{\bullet}$ is in competition with a

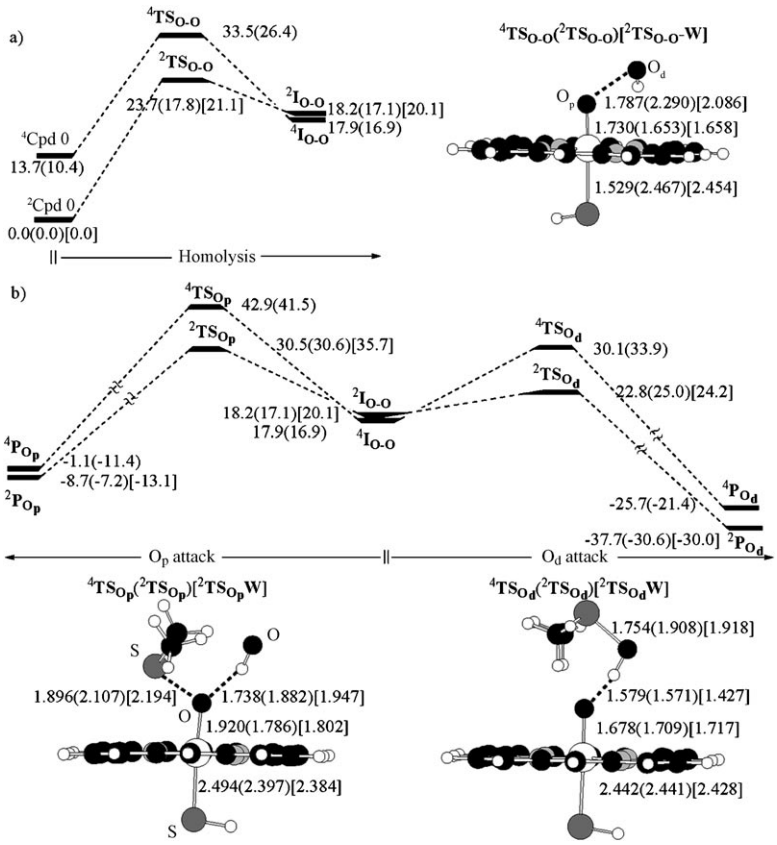

Figure 2. Geometries of key species (B3LYP/LACVP**, bond lengths in Å) for the sulfoxidation of DMS by Cpd 0 by the stepwise homolysis/sulfoxidation mechanism, shown in parts (a) and (b), respectively. The relative energies (in kcal mol^{-1}) of the species are given as $E_1(E_2)[E_{2W}]$, where the E_{2W} data correspond to the effect of an additional single water molecule hydrogen-bonded to the FeOOH moiety.

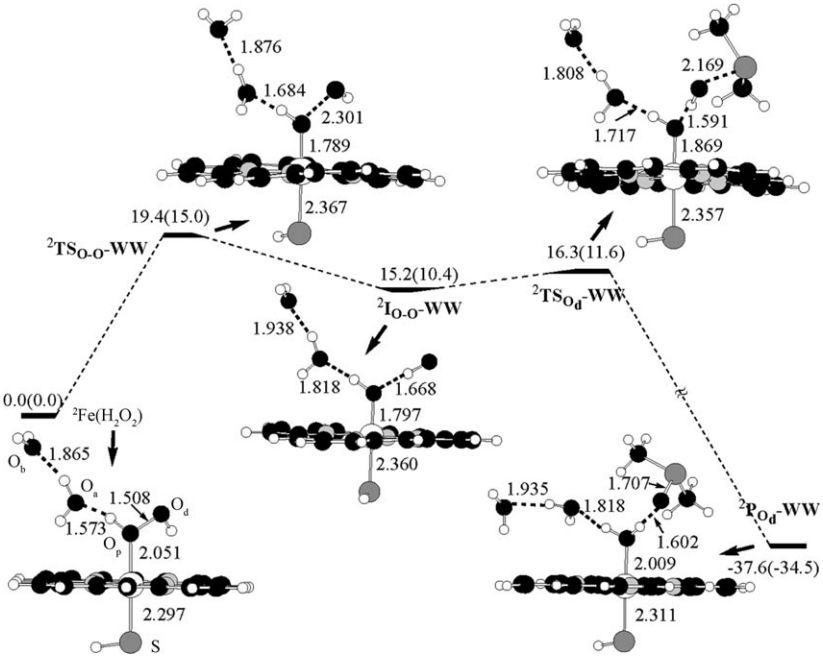

Figure 3. Geometries of key species (B3LYP/LACVP, bond lengths in Å) and energy profiles for the sulfoxidation of DMS assisted by an acidic cluster $H_3O^+(H_2O)$. Relative energies for the species are given (in kcal mol^{-1}) as $E_1'(E_2')$; the primes mean that the geometry is optimized with LACVP.

meso hydroxylation of the porphyrin.[17,18] Because the latter process[17,18,21] has a barrier of 1.3 kcal mol^{-1} while the sulfoxidation barrier here is 7.9 kcal mol^{-1}, it follows that the yield of sulfoxidation by Cpd 0 will be small, whereas in reality the yield is good. With Fe(H_2O_2), the sulfoxidation (Figure 3) will be in competition with the loss of H_2O_2.[3] As the bond-dissociation energy of H_2O_2 in the Fe(H_2O_2) complex is 10.1 kcal mol^{-1} (B3LYP/LACV3P + *// LACVP),[17] while the lowest estimate for the barrier (Figure 3) of O–O homolysis from Fe(H_2O_2) is 15.0 kcal mol^{-1}, this result indicates that with Fe(H_2O_2), H_2O_2 loss will prevail whereas sulfoxidation will be minor, which goes against experimental results.[6,13,14] Another option for Fe(H_2O_2) is the formation of Cpd I by the O–O homolysis/H-abstraction mechanism, which seems to have a small barrier.[22] This step can be followed by fast sulfoxidation via Cpd I, as in Figure 1.

In summary, this study establishes that Cpd I is the most reactive species, and shows that Cpd 0 and the Fe(H_2O_2) complex are inferior oxidants. Thus, in the wild-type enzyme, in which Cpd I is formed through a hybrid homolytic/heterolytic mechanism, the "OH species", generated after the initial O–O cleavage, has no real lifetime to oxidize substrates because it generates Cpd I through barrier-free protonation.[23] The overall barrier for the formation of Cpd I is 12–14 kcal mol^{-1},[23] which means that Cpd 0 will yield Cpd I faster than undergoing bond homolysis and sulfoxidation (Figure 1). According to Figure 1, Cpd I (once formed) will cause fast sulfoxidation as experimentally observed.[6,13–15] By contrast, if as a result of the T→A mutation (for example, T268A in $P450_{BM3}$) the only available oxidant were Cpd 0, one would then expect it to cause sluggish sulfoxidation compared with the native enzyme, whereas in fact, the sulfoxidation is unaffected by the mutation. While these conclusions do not explain the tantalizing results of Cryle and De Voss,[13] they nevertheless rule out a straightforward reactivity of Cpd 0 and suggest new possibilities. For example, since the different enantioselectivity of P450 oxidation is not necessarily a probe of the identity of the oxidant, is it possible that the results of thiafatty acid oxidation are not affected by the mutation of $P450_{BM3}$, simply because thiafatty acids promote efficiently the formation of Cpd I even in the T268A mutant, as opposed to the normal fatty acid substrates, which do not? Can this question be answered by experiment?

Methods Section

All computations were carried out using the B3LYP method. At the highest level, geometries were optimized with the LACVP** basis set followed by energy correction using single-point calculations with LACV3P++** (hence E_1). Bulk polarity was included by using the self-consistent reaction-field method in Jaguar 6.5[24] with a dielectric constant $\varepsilon = 5.7$ and a probe radius of 2.72 Å. The amidic NH···S interactions in the enzyme[25] were modelled[4] by using two NH_3 molecules coordinated to the sulfur ligand. With the addition of zero-point energy, the relative energy is referred to as E_2. In one case (Figure 3) the geometry was optimized with LACVP followed by the same corrections; the corresponding levels are indicated with primes, E_1' and E_2'. The study was performed with Jaguar 6.5 and Gaussian 03.[26]

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