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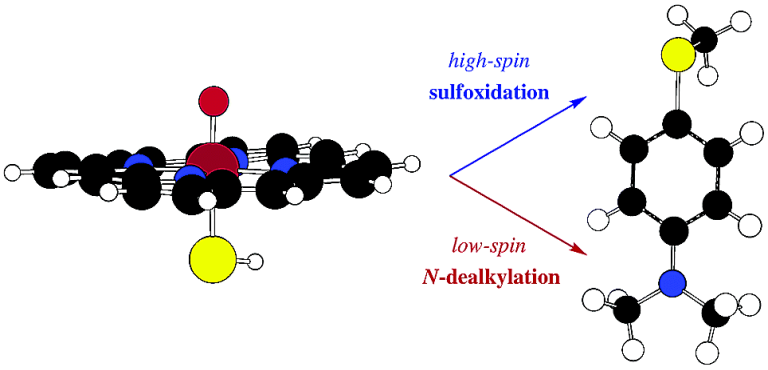

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Can a Single Oxidant with Two Spin States Masquerade as Two Different Oxidants? A Study of the Sulfoxidation Mechanism by Cytochrome P450

Pankaz K. Sharma, Samuël P. de Visser, and Sason Shaik*

Department of Organic Chemistry and the Lise Meitner-Minerva Center for Computational Quantum Chemistry, The Hebrew University of Jerusalem, Givat Ram 91904, Jerusalem, Israel

Received March 13, 2003; E-mail: sason@yfaat.ch.huji.ac.il

The mechanisms of cytochrome P450 reactions still pose an intellectual challenge with tantalizing puzzles.[1] The source of these puzzles is the uncertain identity of the oxidizing species. The principal oxidant is the high-valent iron−oxo porphyrin complex (**1**, Figure 1), known as compound I (Cpd I), and considered by many to be the sole oxidant.[1,2] There exist, however, results that imply the existence of a second oxidizing species. A prime candidate for this species is postulated to be the precursor of Cpd I, the ferric peroxide porphyrin (**4,** Figure 2), so-called Cpd 0.[3] Opinions among the mechanistic chemists have been swaying between these two alternatives for a few years now with no clear resolution.

This exciting dilemma has reemerged from a recent study of the competition between sulfoxidation and N-dealkylation, in the reaction of P450 with dimethyl-(4-methylsulfanyl-phenyl)amine.[4] Thus, a mutation of the threonine residue, known to be involved in the protonation machinery that converts Cpd 0 to Cpd I, increased sulfoxidation over N-dealkylation. However, substituting the C−H's of the *N*-methyl group by deuteriums led to a significant intrinsic kinetic isotope effect, but did not affect the ratio of sulfoxidation to N-dealkylation. Since the threonine mutation increases the yield of Cpd 0, these patterns are consistent with sulfoxidation being mediated mostly, or only, by Cpd 0, while N-dealkylation is mediated exclusively by Cpd I. This two-oxidant reactivity is puzzling, *since Cpd I by itself is able to perform both sulfoxidation*[1,5] *and N-dealkylation*;[1,6] sulfoxidation occurs by direct oxygen transfer,[5] while N-dealkylation occurs either by initial electron transfer or by hydrogen abstraction from the C−H bond of the N-alkyl group.[6] Indeed, Jones and co-workers[4] pointed out that since the mutation of phenyl alanine (F87A), which does not affect the conversion of Cpd 0 to Cpd I, nevertheless reduces the amount of sulfoxidation, the regioselectivity changes may reflect changes in the active site and not in the oxidizing species. Jones and co-workers[4] postulated an alternative scenario with *a regioselective reactivity of the two spin states of Cpd I*, which thereby masquerade as two oxidants. This communication provides computational support for the second postulate; it highlights the experiment−theory synergism and outlines a novel mechanistic direction.

The computations used B3LYP, hybrid density functional,[7a] to study the mechanisms of sulfoxidation of dimethyl sulfoxide (DMS) by Cpd I vis-à-vis Cpd 0. Sulfoxidation by Cpd I was studied with the LACVP**(Fe)/6-31G**(C,H,O,N,S) basis set, in brief LACVP**, followed by single-point calculation with LACV3P^{+}*(Fe)/6-311+G*-(C,H,O,N,S), LACV3P^{+}* in brief. For the reaction of Cpd 0, LACVP(Fe)/6-31G(C,H,O,N,S) optimization followed by a single-point calculation with LACVP** proved sufficient. Optimizations and frequency analyses were done with the GAUSSIAN98.[7b] Interactions of the protein pocket with Cpd I were mimicked as previously,[8,9] and studied with Jaguar 4.1.[7c] More details can be found in the Supporting Information.

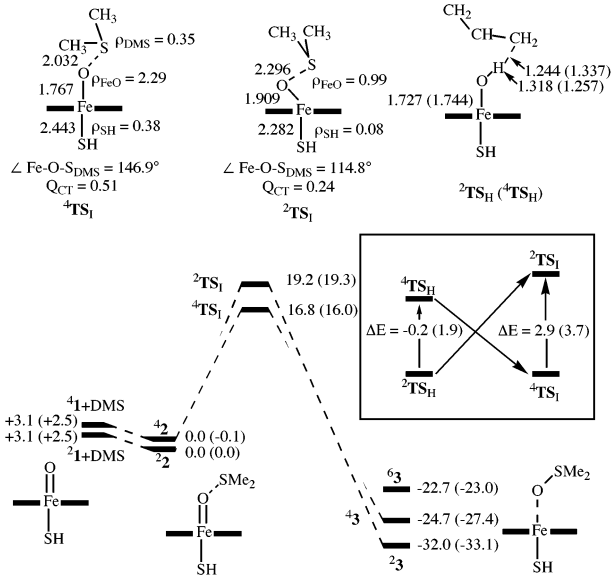

Figure 1. Energy profiles (in kcal/mol) for sulfoxidation of DMS by Cpd I (^{2,4}**1**) via ^{2,4}**TS$_I$**. The relative energies in parentheses correspond to the species under the influence of a dielectric constant ($\epsilon = 5.7$). The ρ values on the structures are group spin densities, while Q_{CT} corresponds to the degree of charge transfer from DMS to Cpd I. The geometries of ^{2,4}**TS$_H$**, the transition states for allylic hydroxylation (ref 9) are also displayed. The inset shows relative energies (including ZPE) for the two spin states of **TS$_H$** vis-à-vis **TS$_I$** for the bare species (out of parentheses) and for the species under the influence of a dielectric constant, $\epsilon = 5.7$ (in parentheses).

Figure 1 shows the energy profiles for sulfoxidation by the doublet and quartet states of Cpd I; both exhibit synchronous oxygen transfer, in which the high-spin (HS) transition state (TS) is significantly lower than the low-spin (LS). Inclusion of NH- - -S hydrogen bonding does not affect the relative energy of the TSs, whereas charge polarization by a nonpolar environment ($\epsilon = 5.7$) increases their gap. Single-point calculation with LACV3P^{+}*, predicts a larger gap of 5.5 kcal/mol. Clearly, in contrast to alkane hydroxylation, where the hydrogen abstraction step has a lower barrier on the LS surface (^{2}**TS$_H$** below ^{4}**TS$_H$**, inset in Figure 2),[9] sulfoxidation has a significantly lower HS barrier. *This indicates that sulfoxidation and N-dealkylation (via C−H hydroxylation) will proceed largely via different spin states of Cpd I.*

The origins of this difference are mechanistic. C−H hydroxylation is stepwise,[1,2,9] and so is the electronic transformation.[9] In the H-abstraction step, both ^{2}**TS$_H$** and ^{4}**TS$_H$** possess similar electronic structures with the exception of the direction of the developing spin on the alkyl radical. As such, in the gas phase ^{4}**TS$_H$** and ^{2}**TS$_H$** are virtually degenerate,[9] and it's the NH- - -S hydrogen bonds and polarity effects that lower ^{2}**TS$_H$** below ^{4}**TS$_H$**.[9] By contrast, sulfoxidation is concerted and so must be the electronic reorganization. Since the products ^{2}**3** and ^{4}**3** have different electronic

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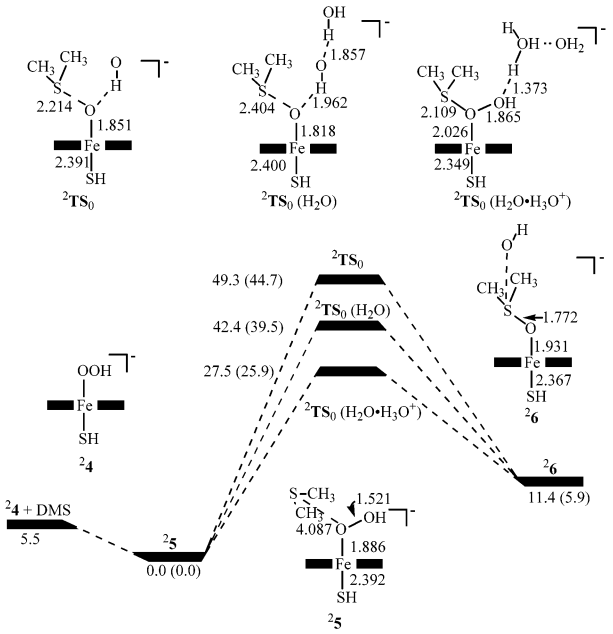

Figure 2. Energy profiles for sulfoxidation of DMS by Cpd 0 (^{2}**4**), via ^{2}**TS$_0$** for the unassisted process, or via ^{2}**TS$_0$** (H_2O) and ^{2}**TS$_0$** ($H_2O{\cdot}H_3O^+$) for the processes assisted by water and the cluster $H_2O{\cdot}H_3O^+$. The corresponding barrier heights (kcal/mol) are indicated near the TS species. The barriers in parentheses include the effect of a dielectric constant (ϵ = 5.7).

structures, ^{2}**TS$_I$** and ^{4}**TS$_I$** will necessarily differ in their key orbitals interactions. Indeed, the wide Fe−O−S_{DMS} angle of 147° in ^{4}**TS$_I$** shows that the bonding between the Cpd I and DMS moieties is mediated by the overlap of the sulfur lone pair orbital of DMS with the vacant $\sigma^*(d_{z^2})$ orbital of iron−oxo. By contrast, the narrow Fe−O−S_{DMS} angle of 115° in ^{2}**TS$_I$** indicates a dominant overlap of the sulfur lone-pair with the singly occupied π^*(FeO) orbital. Since two-orbital−two-electron interaction is more stabilizing than three-electron interaction, ^{4}**TS$_I$** ends up having stronger bonding and greater stability than ^{2}**TS$_I$**. This excess stabilization is apparent from the higher degree of charge transfer (Q_{CT}), the shorter S_{DMS}-O distance in ^{4}**TS$_I$**, and the more deformed iron−oxo moiety in ^{2}**TS$_I$** where the Fe−O bond bends toward the porphyrin plane.

Figure 2 displays the computed profile for Cpd 0 reacting with DMS. The reaction involves a concerted nucleophilic displacement with departure of OH^- from Cpd 0. The barrier for this reaction is 49.3 kcal/mol and decreases to 44.7 kcal/mol in the presence of a polarizing field. The effects of acid catalysis by putative "acids", in the protein pocket, were studied for two limiting situations: The first, and the more likely one, involves a single water molecule hydrogen-bonded to the departing OH^-, and the second, to a $H_2O{\cdot}(H_3O^+)$ cluster that mimics an extreme situation of excess protons in the pocket. Allowing the geometries of the systems to relax[10] led to two new transition states, ^{2}**TS$_0$**(H_2O) and ^{2}**TS$_0$**($H_2O{\cdot}H_3O^+$). The first one, ^{2}**TS$_0$**(H_2O), has a barrier of 42.4 (39.5; ϵ =5.7) kcal/mol. The second, ^{2}**TS$_0$**($H_2O{\cdot}H_3O^+$), has a barrier of 27.5 (25.9, ϵ = 5.7) kcal/mol and involves simultaneous sulfoxidation and a Grotthuss-type protonation mechanism[10a] of the departing hydroxide by the acidic cluster. Thus, even in the presence of a potent acid catalysis, which is anyway unrealistic for the mutant since it lacks an efficient protonation machinery, sulfoxidation by Cpd 0 has a much higher barrier than Cpd I. This was found also for ethene epoxidation[11] and is experimentally supported.[3c]

In conclusion, the hypothesis that sulfoxidation is affected by Cpd 0, whereas N-dealkylation (via C−H abstraction), by Cpd I, is not supported by the calculations. The results favor the alternative scenario, namely *that Cpd I leads to both sulfoxidation and N-dealkylation via a regioselective spin-state reactivity that is modulated by polarity and hydrogen-bonding factors*.[9] Indeed, the results of Jones and co-workers[4] with the F87A mutant further indicate that changes that affect the topography of the protein pocket affect also the regioselectivity of Cpd I. Under a premise of slow spin crossover in the enzyme−substrate (ES) complexes, the ^{4}ES(sulfox) and ^{2}ES(N-dealk) will not interconvert.[4,12] As such, *the two states of a single oxidant, Cpd I, could behave as two different oxidant species*. The availability of multiple protonation pathways[10] that convert Cpd 0 to Cpd I and produce different yields of spin species of the latter could be considered a scenario whereby threonine mutation affects the regioselectivity patterns observed by Jones and co-workers.[4]

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Supporting Information Available: Computational data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Since the spin states of Cpd I (in the protein pocket) differ solely by the direction of the spin on the porphyrin, spin−orbit coupling will be weak.

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