Energetics for the Oxygen Rebound Mechanism of Alkane Hydroxylation by the Iron–Oxo Species of Cytochrome P450

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Density-functional-theory calculational results on the reaction pathway for alkane hydroxylation by a compound I model of cytochrome P450 are discussed. Our calculations demonstrate that the transition state for the H-atom abstraction of ethane involves a linear (Fe)O···H···C array and that the resultant carbon radical is bound to the iron–hydroxo species. This comptational result is partly consistent with the oxygen rebound mechanism in that the direct H-atom abstraction by the iron–oxo species takes place in the initial stages of the reaction pathway. However, the iron–hydroxo species cannot be viewed as a stable reaction intermediate in view of the energy diagram. Our results may not be consistent with the model of a free radical species with a finite lifetime and barrier to displacement of the OH group from the iron center that is commonly assumed and typically stated for the oxygen rebound mechanism.

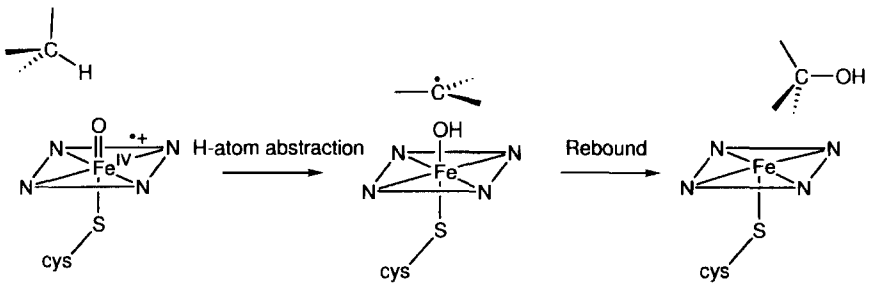
Cytochrome P450 enzymes are a group of monooxygenase enzymes that oxygenate a wide variety of substrates under physiological conditions.[1] The iron–oxo species called compound I, which is assumed as Fe^{IV}=O($Por^{\bullet+}$) or Fe^{V}=O-(Por), is the key intermediate that is the most likely candidate for the oxygen source of P450, in which Por is porphyrin. Alkane hydroxylation, which is an example of such oxygenation reactions by P450, is widely believed to occur by a mechanism involving a direct H-atom abstraction from a substrate, followed by a rapid transfer of metal-bound hydroxyl radical to an intermediate alkyl radical, as indicated in Scheme 1. Strong support for this mechanism, called the oxygen rebound mechanism, has come from the functional P450 models developed by Groves and co-workers;[2] it has been supported by accumulated experimental results of stereochemistry, regiochemistry, and isotope effect studies.[1] Theoretical studies have explored the electronic structures of model iron–porphyrin systems with CH_3S^- as the proximal ligand that focus P450.[3] Recently, Shaik et al. investigated some mechanistic aspects of P450-catalyzed hydroxylations and indicated the participation of two-state reactivity in the C–H bond dissociation of substrate and in the resultant iron–hydroxo intermediate.[4] Crabtree and Siegbahn studied the mechanism and energetics for the H atom abstraction of methane using a small model.[5] Detailed calculations on the reaction pathway and energetics for the oxygen rebound mechanism are crucial to increase our knowledge of the functions of P450, especially of the H-atom abstraction.

Method of Calculation

The compound I model we used in this study is a six-coordinate iron–oxo species $Fe^{+4}O^{-2}(C_{20}N_4H_{12})^{-1}(SCH_3)^{-1}$; thus the total charge of this model is zero. The method of choice is the hybrid Hartree–Fock/density functional theory (DFT) B3LYP method,[6,7] which has been successfully applied to many catalytic and enzymatic reactions. For the Fe atom we used the triple-zeta-valence (TZV) basis set,[8] and for the H, C, N, O, and S atoms we used the D95 double-zeta basis set.[9] Geometry optimizations were performed in the doublet, quartet, and sextet states with respect to the reaction intermediates and the transition states without imposing symmetry restriction; however, vibrational frequency calculations, which are desirable to confirm that the optimized geometries are truly minimum points or saddle points on the potential energy surface, were not carried out due to the high computational demands of the large model. All DFT calculations were performed with the Gaussian 98 package.[10]

Results and Discussion

Figure 1 shows the reaction intermediates and the tran-



Scheme 1.

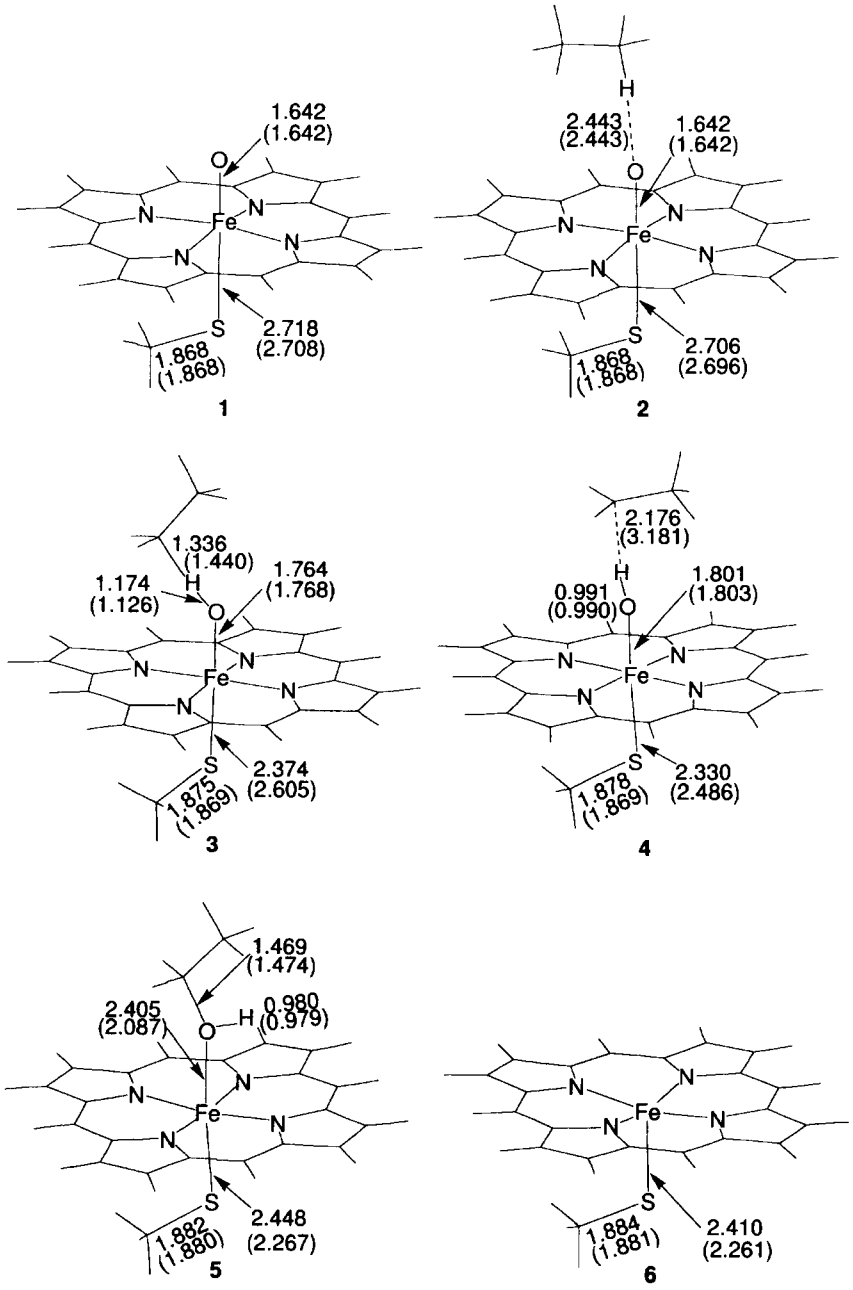


Fig. 1. Optimized geometries of the reaction species in the hydroxylation of ethane by the compound I model of the quartet and doublet states. Bond distances in parentheses are for the doublet state. Values in units of Å.

sition state for the conversion of ethane to ethanol by the compound I model of the spin quartet and doublet states. Let us look at the geometries of the quartet state because it plays an important role in the course of the reaction. The iron–oxo model (**1**) has an Fe–O bond of 1.642 Å and a very long Fe–S "bond" of 2.718 Å in the quartet state; the essential structural features are in good agreement with those in the recent study.[3d] The long Fe–S bond is not an artifact of our DFT calculation using the CH_3S^- ligand; this was reproduced in a different model with a proximal cysteinate ligand. In the initial stages of the reaction, the substrate comes into contact with the iron–oxo model to form the reactant complex (**2**). The transition state (**3**) for the H-atom abstraction has an O–H bond of 1.174 Å and a C–H bond of 1.336 Å these bond distances as well as the C–H–O–Fe arrangement are reasonable for this electronic process. Interestingly, the Fe–S bond is significantly decreased from 2.706 Å in **2** to 2.374 Å in **3**. The electronic process for the H-atom abstraction is in good agreement with what the oxygen rebound mechanism suggests. However, since the produced "ethyl radical" is bound to the active center of the complex, this mechanism cannot be viewed as a free radical process. This iron–hydroxo species (**4**) is converted to the product complex (**5**) that involves the product ethanol as a ligand. The final complex (**6**) after the release of the alcohol product remains almost unchanged from the heme moiety of **5**. Calculated

charges and spin densities for the Fe and O atoms and the CH_3S and porphyrin moieties are listed in Table 1. The calculated charge (and spin density) of the "ethyl radical" in **4** are 0.1 (1.0) and 0.0 (1.0) in the doublet and quartet states, respectively, and therefore it is a neutral radical species.

Figure 2 shows that the quartet state should provide a low-cost reaction pathway for the hydroxylation. The doublet and quartet potential energy surfaces are closely lying in the entrance channel, i.e., in the vicinity of **1** and **2**; however, the quartet state lies 6.4 kcal/mol below the doublet state in **3**. When we follow the quartet potential energy surface, the C–H bond dissociation should require 22 kcal/mol. This value is rather high for an enzymatic reaction, but is in good agreement with the value for the H-atom abstraction of methane by the bare FeO^+ complex.[11] The resultant iron–hydroxo species **4** that binds "ethyl radical" exists as a stable point in a mathematical sense on the potential energy surface. However, we could not find the transition state that correctly connects **4** and **5**.[12] Therefore the barrier height for the transition state should be low if it even exists on the reaction pathway. The use of dotted lines in Fig. 2 shows that further theoretical considerations are required to better understand the rebound step. Although the molecular system should pass through this species in the course of alkane hydroxylation, it cannot be viewed as a stable reaction intermediate. Our results may not be consistent with the model of a radical intermediate with a finite lifetime and barrier to displacement of the OH group from the iron center that is commonly assumed for the oxygen rebound mechanism. It is important to perform vibrational frequency calculations to increase our knowledge on this important "intermediate" with respect to the question of whether the radical is virtually free or not. We found that the three spin states are nearly degenerate in the exit channel, i.e., in **5** and **6**. In view of the energy diagrams, both doublet and quartet spin states are responsible for the hydroxylation reaction; the two-state reactivity paradigm[13] is significant as in the similar gas-phase electronic processes in the FeO^+/H_2[14] and FeO^+/CH_4[11] systems.

Table 1. Calculated Mulliken Charges and Spin Densities[a] for the Fe and O Atoms and the CH_3S and Porphyrin (Por) Moieties

	Doublet state				Quartet state			
	Fe	O	CH_3S	Por	Fe	O	CH_3S	Por
1	1.1(1.2)	−0.4(0.9)	0.0(−0.8)	−0.7(−0.3)	1.1(1.2)	−0.4(0.9)	0.0(0.8)	−0.7(0.1)
2	1.1(1.2)	−0.4(0.9)	0.0(−0.8)	−0.7(−0.3)	1.1(1.2)	−0.4(0.9)	0.0(0.8)	−0.7(0.1)
3	1.1(0.8)	−0.2(0.6)	0.0(−0.7)	−0.8(−0.3)	1.1(1.4)	−0.2(0.6)	−0.1(0.4)	−0.6(0.0)
4	1.1(0.9)	−0.3(0.1)	0.0(−0.7)	−0.9(−0.3)	1.1(1.8)	−0.3(0.3)	−0.1(0.1)	−0.7(−0.2)
5	1.1(1.0)	−0.2(0.0)	−0.2(0.1)	−1.0(−0.1)	1.2(2.6)	−0.2(0.0)	−0.3(0.5)	−0.9(−0.1)
6	1.0(1.2)	-	−0.2(−0.1)	−0.8(−0.1)	1.2(2.5)	-	−0.4(0.5)	−0.8(0.0)

a) The values in parentheses are spin densities.

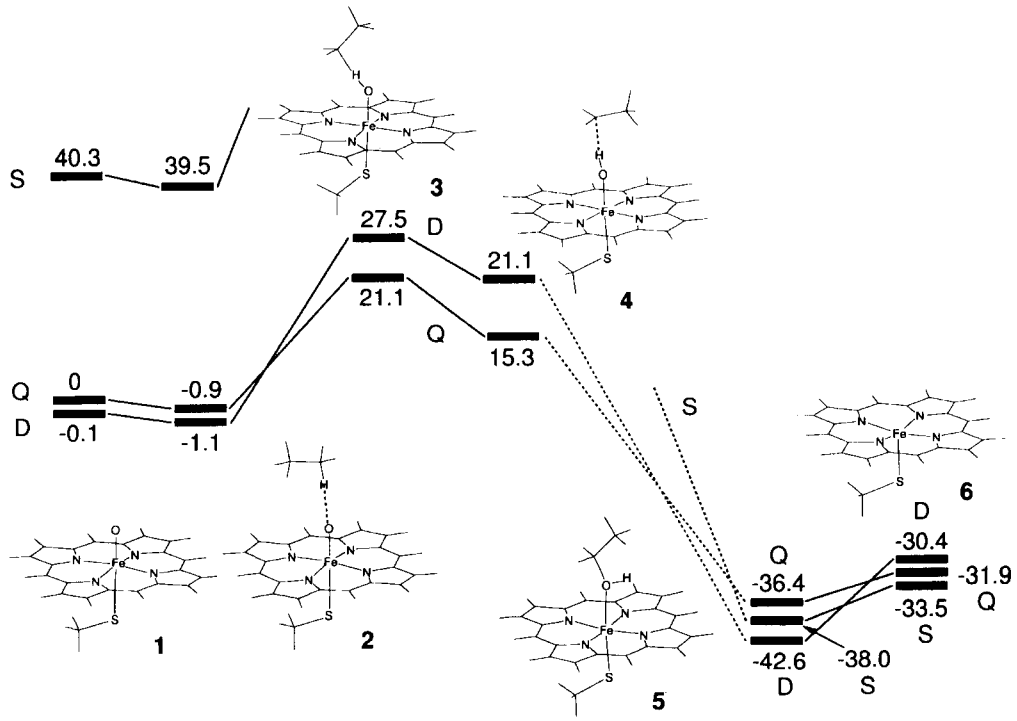


Fig. 2. Energy diagrams for the hydroxylation of ethane by the compound I model in the spin doublet, quartet, and sextet states. Values in units of kcal mol^{-1}.

Recently Shaik and co-workers found the transition state for the rebound step on the quartet potential energy surface using a compound I model with SH^- as the proximal ligand, the transition state lying 5 kcal/mol above the iron–hydroxo species, whereas they could not find any transtion state on the doublet potential energy surface.[15] Thus, our knowledge on the mechanism of cytochrome P450 is still limited and further theoretical calculations are necessary to better understand the important enzymatic function.

A recent subject of considerable research effort in soluble methane monooxygenase (sMMO)- and P450-catalyzed hydroxylations is whether both hydroxylation mechanisms involve the formation of carbon radicals as an intermediate species or not.[16] As mentioned earlier, strong support for the radical mechanism has come from experimental results of stereochemistry, regiochemistry, and isotope effect studies.[1,2] In contrast, attempts by Newcomb et al. to determine the lifetime of the intermediate by the use of highly reactive "radical clock" substrates indicated that the mechanistic paradigm for P450-catalyzed hydroxylations is not complete.[17] We have also been critical to the radical mechanism in methane hydroxylation by sMMO and have proposed a concerted mechanism in which a coordinatively unsaturated iron active center should play an essential role in substrate binding, H-atom abstraction, and rebound steps.[18] If the porphyrin ring is appropriately bent in the course of the reaction, our concerted mechanism should apply to P450-catalyzed hydroxylations.[19] However, since the approach of substrate to the enzyme heme iron is difficult due to the steric problem and bending of the porphyrin ring should require high cost of energy, the oxygen rebound mechanism might be a reasonable hypothesis for P450-catalyzed hydroxylations of hydrocarbons in some aspects *if the active species of P450 is truly of the compound I type, a six-coordinate iron–oxo species.* We conclude that the coordination environment of the active species determines how the enzymatic hydroxylations occur in the initial stages. We will continue mechanistic studies to increase our knowledge on the two different mechanisms and will report detailed results in due course.

Concluding Remarks

We presented new DFT results on the reaction pathway for the alkane hydroxylation by a compound I model of cytochrome P450 at the B3LYP level of theory, especially on H-atom abstraction. The doublet and quartet potential energy surfaces are closely together in the entrance channel; however, the quartet state lies 6.4 kcal/mol below the doublet state in the transition state for the H-atom abstraction step. Our calculations demonstrated that the transition state for the H-atom abstraction of ethane involves a linear (Fe)-O···H···C array and that the resultant carbon radical is bound to the iron–hydroxo species. This process is partly consistent with the oxygen rebound mechanism in that the direct H-atom abstraction by the iron–oxo species takes place in the initial stages of the reaction pathway. However, we could not find the transition state that connects the iron–hydroxo species and the final complex including the product alcohol as a ligand. Thus, it cannot be viewed as a stable reaction intermediate. Our results may not be consistent with the model of a free radical intermediate with a finite lifetime and barrier to displacement of the OH group from the iron center that is commonly assumed for the oxygen rebound mechanism.

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