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Two-Step Concerted Mechanism for the Hydrocarbon Hydroxylation by Cytochrome P450

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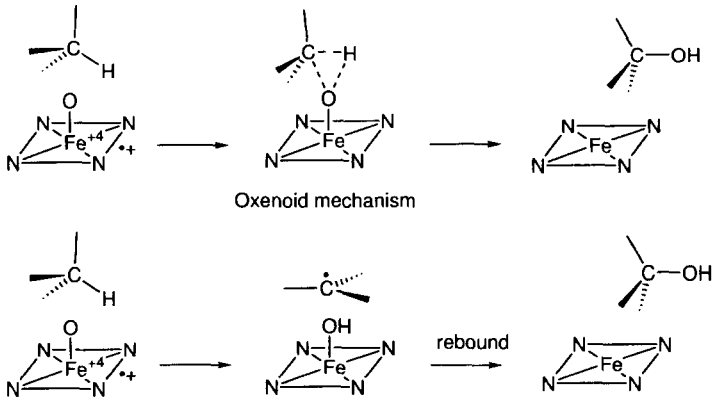
A possible two-step mechanism for the hydrocarbon hydroxylation by the oxoferryl active species of cytochrome P450 is discussed. We propose that the catalytic reaction is initiated by the formation of a reactant complex involving a five-coordinate carbon species or a σ-complex, followed by concerted hydrogen and alkyl migrations which lead to the formation of a product complex involving alcohol as a ligand. The porphyrin ring is predicted from molecular mechanics and extended Hückel calculations to be significantly bent in the reactant complex and in the hydroxy intermediate to form an energetically preferred octahedral environment at the iron active center. Such bent structures for the porphyrin ring are consistent with observed *cis*-coordination geometries for the M(porphyrin)L_2 complexes, in which M is a large transition-metal ion and L is a ligand. We suggest that possible inversion at the five-coordinate carbon species in the reactant complex can occur to lead to partial inversion of stereochemistry at a labeled carbon.

Hydrocarbons are biologically converted into alcohols by cytochrome P450[1,2] and methane monooxygenase (MMO)[3—6] under physiological conditions. The active sites of these metalloenzymes toward substrate hydrocarbons are proposed to contain one or two oxoferryl species. As shown in the upper illustration of Scheme 1, the mechanism of P450-catalyzed hydroxylations was initially proposed to follow a concerted pathway, "oxenoid mechanism", based on observed retention of stereochemistry and small kinetic isotope effects.[1] Nowadays hydroxylations by cytochrome P450 are 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 (Scheme 1, bottom). This widely-believed radical mechanism is called "oxygen rebound mechanism";[7] it has been supported by accumulated experimental results of stereochemistry, regiochemistry, and isotope effect.

Recent studies of Newcomb, Hollenberg, and their collaborators[8] have suggested from an estimated short radical lifetime of less than 100 fs that the consensus hydroxylation mechanism on the basis of the radical mechanism in cytochrome P450 is incomplete or incorrect. Their results are inconsistent with a long-lived free-radical intermediate in P450-catalyzed hydroxylations. A side-on approach of oxygen to a C–H bond is suggested, as opposed to the linear C–H–O array of a conventional H atom abstraction.[7]

As indicated by Schröder, Schwarz, and their collaborators,[9—11] the gas-phase methane-methanol conversion by the bare FeO^+ complex may be viewed as a simple model for hydrocarbon hydroxylations by various catalytic and enzymatic systems. Thus a detailed analysis for the reaction pathway is the key to better understanding of closely-



Scheme 1.

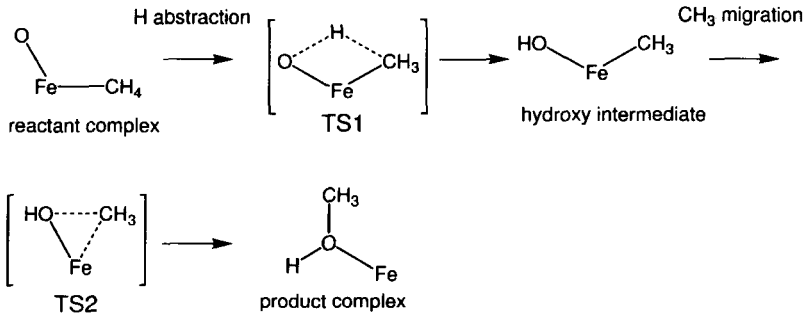
related catalytic and enzymatic hydrocarbon hydroxylations. We have proposed from density-functional-theory (DFT) calculations that a two-step concerted mechanism can reasonably explain the general features of the reaction of methane with MnO^+, FeO^+, and CoO^+.[12,13] Our finding is that the energetically preferred reaction pathway should involve two transition states (TS1 and TS2), as shown in Scheme 2. The two barrier heights for the four-centered TS1 and the three-centered TS2 determine the general features of the reaction efficiency and the product branching ratio, respectively.[13] In addition, we recently confirmed that the four-centered TS1 lies in energy below the transition state (for a direct H-atom abstraction) that has a linear C–H–O array.[14]

We have also been interested in the activation of dioxygen and methane on various dinuclear iron models of soluble MMO.[15—21] Recently we confirmed from DFT calculations that a hydrogen abstraction of methane occurs reasonably well on a diiron model complex of soluble MMO via a four-centered transition state similar to TS1 in Scheme 1[18] and that the entire reaction profile of the methane hydroxylation on the diiron model is essentially identical to that of the gas-phase methane–methanol conversion.[19] In the initial stage of our mechanism, the iron of the reactive oxoferryl species and a substrate hydrocarbon come into contact to form a reactant complex, a kind of σ-complex. The C–H bonds of the substrate are significantly activated by electron transfer from the HOMO of hydrocarbon to the d-block orbitals of complex.[16] We think that these arguments are relevant for the hydrocarbon hydroxylation catalyzed by cytochrome P450. Our main purpose in this article is to look at whether or not the two-step concerted mechanism is applicable to the enzymatic reaction under debate.

Two-Step Concerted Mechanism for Cytochrome P450. We believe through our previous work that the oxoferryl "iron" is an important reaction center throughout the entire reaction pathway rather than the oxoferryl "oxygen". If a coordinatively unsaturated oxoferryl species is created in enzymatic systems, our two-step concerted mechanism in Scheme 2 can play a role in various biological hydroxylations. From the viewpoint of known catalytic chemistry, it seems to be reasonable to assume that the metal active center of enzyme should be coordinatively unsaturated. We thus think that the iron active center of cytochrome P450 should lack or be apart from the axial cysteinyl ligand although we take cognizance of an important "push" effect of the proximal thiolate ligand that is proposed to play a role for cleavage of the O–O bond of a hydroperoxide complex generating compound I.[2]

Under the working hypothesis that the iron active species of cytochrome P450 is a five coordinate oxo(porphyrinato)-iron(IV), we propose a possible two-step concerted mechanism for the hydrocarbon hydroxylation catalyzed by P450, following the reaction pathway indicated in Scheme 1. Let us now consider the P450-catalyzed benzylic hydroxylation of ethylbenzene. McMahon et al.[22] first reported retention of stereochemistry at the benzyl carbon in this reaction, whereas White et al.[23] reported 23—40% inversion of stereochemistry. To show plausible geometries for the reactant complex, the hydroxy intermediate, and the product complex, we performed molecular mechanics (MM) calculations based on the Universal force field (UFF).[24,25] We show in Fig. 1 optimized structures of these species. These structures are, of course, similar to those of the gas-phase[12—14] and the MMO-catalyzed[18,19] methane hydroxylations in their essential features around the metal active center.

The iron in the reactant complex and the hydroxy intermediate was computed to be in a nearly octahedral environment, and as a consequence the porphyrin ring is significantly deformed from a plane. We think that these geometries are reasonable from the viewpoint of general features of the coordination sphere of Fe ions, which in general favor an octahedral environment. Closely-related *cis*-coordination geometries were recently characterized in the X-ray structural analyses of M(porphyrin)L_2 complexes by Brand and Arnold,[26] in which M is a large transition-metal ion such as Zr, Hf, Nb, or Ta and L is a ligand. A similar strained structure was also obtained from MM calculations as one of the most probable conformations of transition metal tetraaza macrocycle complexes.[27] Thus, such computational results are not surprising. The bending angle of the porphyrin ring in the reactant complex was calculated to be 98.7°. The five-coordinate carbon species of the substrate exhibits an η^2-binding mode, as we expect. The H–C–H angle of the substrate ethylbenzene was computed to be significantly opened up to 145.2°, which is in good agreement with an observed B–C–B angle of 149.3° in a Zr complex that involves a five-coordinate carbon species.[28] Low-temperature reactions of atomic cobalt with CH_4 and CD_4 to form the σ complexes were reported by Billups et al.;[29] their FTIR matrix isolation spectroscopy in solid argon demonstrated that the methane is



Scheme 2.

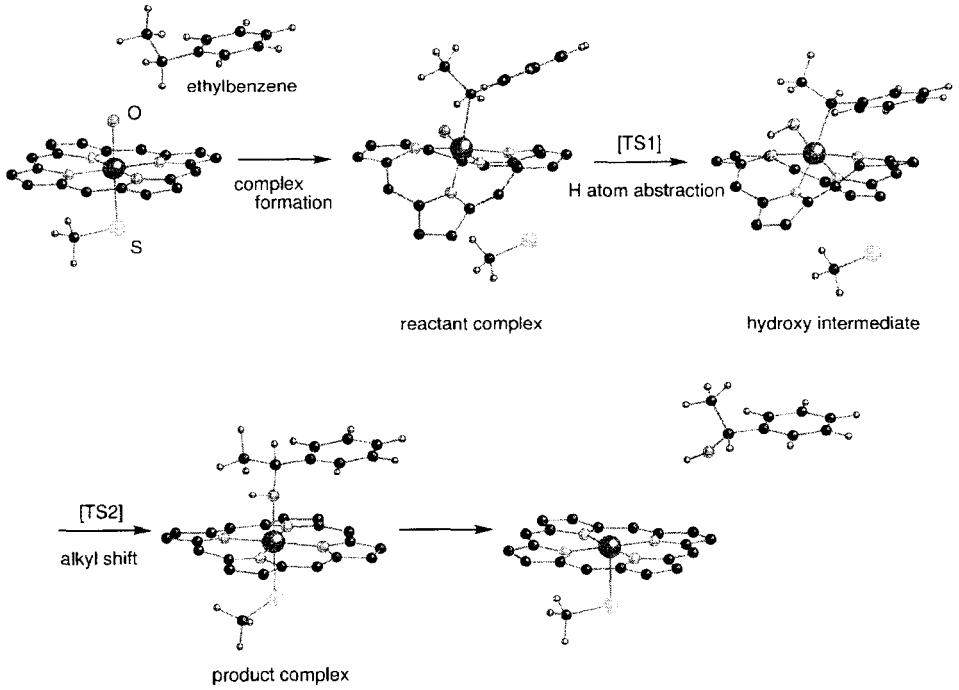


Fig. 1. A possible two-step concerted mechanism for the benzylic hydroxylation by a supposed active species of cytochrome P450.

significantly distorted on the complexes. We think that in the near future a lot of examples of such five-coordinate carbon species or σ complexes will be found in actual catalytic and enzymatic systems using modern spectroscopic techniques.

The reader may still suspect that the porphyrin ring is bent from the well-known planar structure both in the reactant complex and in the hydroxy intermediate. However, this view is reasonable from a theoretical point of view.[30,31] To find a good reason for this, we computed an energy change along such a distortion with the extended Hückel method.[32] This method is reliable for bond angles and dihedral angles in organic and inorganic molecular systems although this approximate molecular orbital method includes no explicit electron–electron interactions. Let us assume that the active species of P450 involves an Fe(IV)–oxo porphyrin cation radical (total charge +3 in our model).[1] A computed potential energy diagram is shown along a reaction coordinate for the bending of the porphyrin ring, i.e., the angle θ indicated in the illustration of Fig. 2. Interestingly, the computed potential energy curve appears to be minimum at θ = 112°, being qualitatively consistent with the MM result described above. We thus predict from qualitative calculations that the porphyrin ring in the reactant complex should exhibit a bent structure. This would be a natural extension of our mechanistic proposal on the methane hydroxylation by FeO^+ and soluble MMO.[21]

Let us next look at orbital interactions between a five-coordinate oxoferryl species and methane, although known cytochrome P450s cannot catalyze the hydroxylation of methane. An orbital interaction diagram for a model complex ($Fe^{+4}O^{-2}[(NH_2^-)_4]^{-3}$; total charge −1) and a D_{2d}-distorted methane is demonstrated in Fig. 3. The fragment molecular orbitals (FMOs) of methane are shown at the right, the FMOs of the complex at the left, and the orbitals reconstructed from these FMOs at the center. As a consequence of the formation of an Fe–C bond as well as Fe–H bonds in the reactant complex, C–H bonds are significantly weakened through two types of important orbital interactions.[16,33] One is the interaction between the HOMO (highest occupied molecular orbital) of alkane (C–H bonding) and the unfilled d orbitals of the complex, and the other is the interaction between the LUMO (lowest unoccupied molecular orbital) (C–H antibonding) and the filled d orbitals of complex. The most important interaction in Fig. 3 is that between the b_2 HOMO of a D_{2d}-distorted methane and the unoccupied nonbonding d orbital at −10.5 eV.

We should state that our concerted mechanism is not a revival of the earlier concerted one, "oxenoid mechanism" (Scheme 1, top), in which the initially formed species (or transition state?) is assumed to involve an O–C bond as well as an O–H bond. We illustrate in Scheme 3 a possible concerted reaction pathway for a hydroxylation by the oxoferryl active species of cytochrome P450. At present we have no information about the structures of TS1 for an H atom abstraction and TS2 for an alkyl migration on the reaction pathway indicated. However, such transition-state structures may be trivial under the working hypothesis that the reaction pathway in Scheme 2 is relevant to the hydrocarbon hydroxylation by P450.

Comparison with Experiments. As mentioned above in the introduction of this paper, Newcomb, Hollenberg, and their collaborators[8] inferred in their "nonsynchronous concerted" mechanism that a hydrogen atom abstraction does not occur with a linear C–H–O array, which is assumed in the widely-believed oxygen rebound mechanism, but via a side-on approach of oxygen to a C–H bond like in the

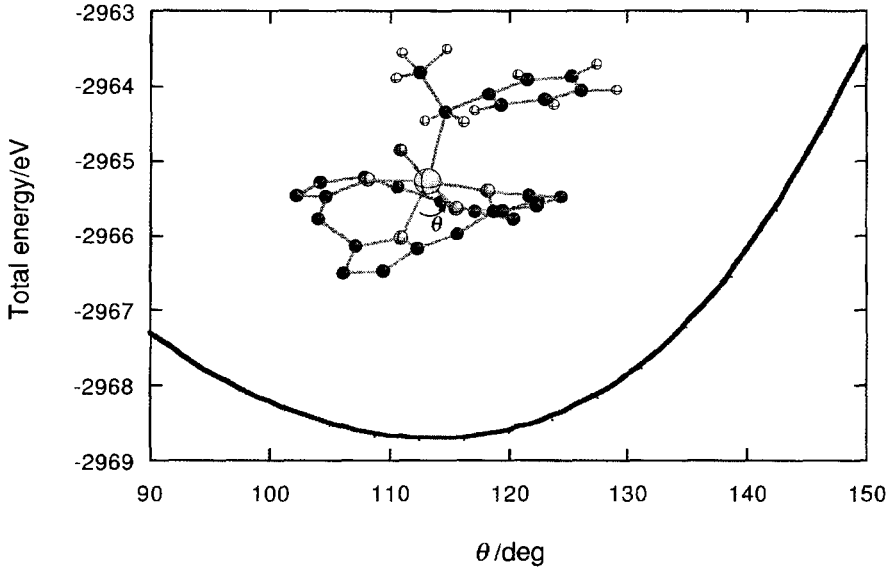
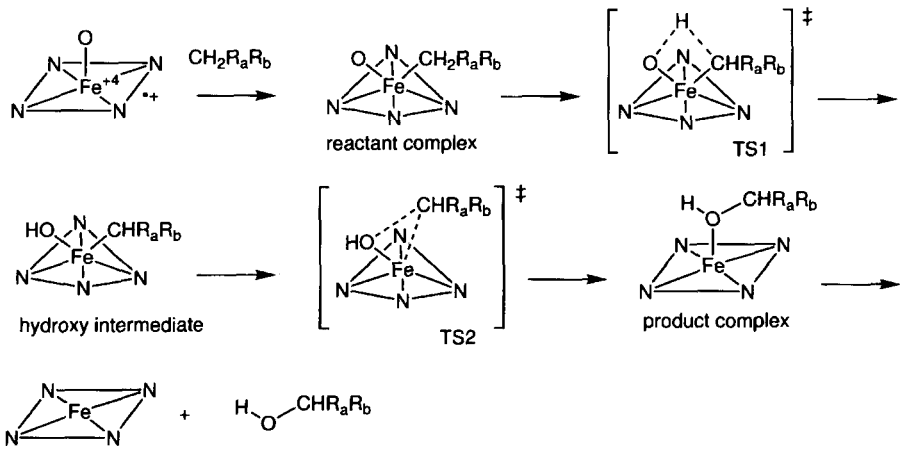


Fig. 2. Total energy diagram along a distortion of the porphyrin ring in the reactant complex.



Scheme 3.

oxenoid mechanism in Scheme 1. Their proposal is similar to those proposed by Shteinman[34] and by Shestakov and Shilov.[35] Our mechanism is different from these; it is important to state that our mechanism includes the formation of a reactant complex involving a five-coordinate carbon species, followed by concerted hydrogen and alkyl migrations that lead to the formation of a product complex involving alcohol as a ligand.

The relative reactivity of C–H bonds in enzymatic hydroxylations has been reported to generally decline in the order of *secondary* > *tertiary* > *primary*.[36] According to a recent review,[37] known C–H bond activations at a transition-metal center in both homogeneous and heterogeneous phases occur preferentially at a primary C–H bond of substrates, in the order of *primary* > *secondary* > *tertiary*. Moreover, recent regioselective study using methylcubane as a substrate has shown that the enzymatic hydroxylations by cytochrome P450 and soluble MMO differ from the radical-based hydroxylation by *t*-BuO· in the product branching ratios.[38] These observations are contrary to the order of reactivity in conventional metal-free C–H bond activations with radicals or strong acids, in which *tertiary* C–H bonds are most reactive. This fact suggests that steric effects at a transition-metal center should have a certain influence on the reactivity of C–H bond activation in the hydroxylations by these metalloenzymes. We think that the reactivity of metal-catalyzed C–H activation would be a function of both C–H bond strength itself and steric effect; thus the regioselectivity of enzymatic hydroxylations[36] is different from that of radical-based hydroxylations in which C–H bond strength itself is a dominant factor.

The reader may think that our concerted mechanism will always afford retention of stereochemistry at a carbon center, inconsistently with experiments. However, if inversion of configuration at a five-coordinate carbon species occurs in a reactant complex, our mechanism can reasonably explain observed partial inversion of stereochemistry at a labeled carbon. In a five-coordinate carbon species with an η^2-mode, inversion at a five-coordinate carbon species is possible, as shown in Scheme 4.[20,21]

Inversion of free methane has been extensively studied by several groups at different levels of theory.[39–41] Since in a re-

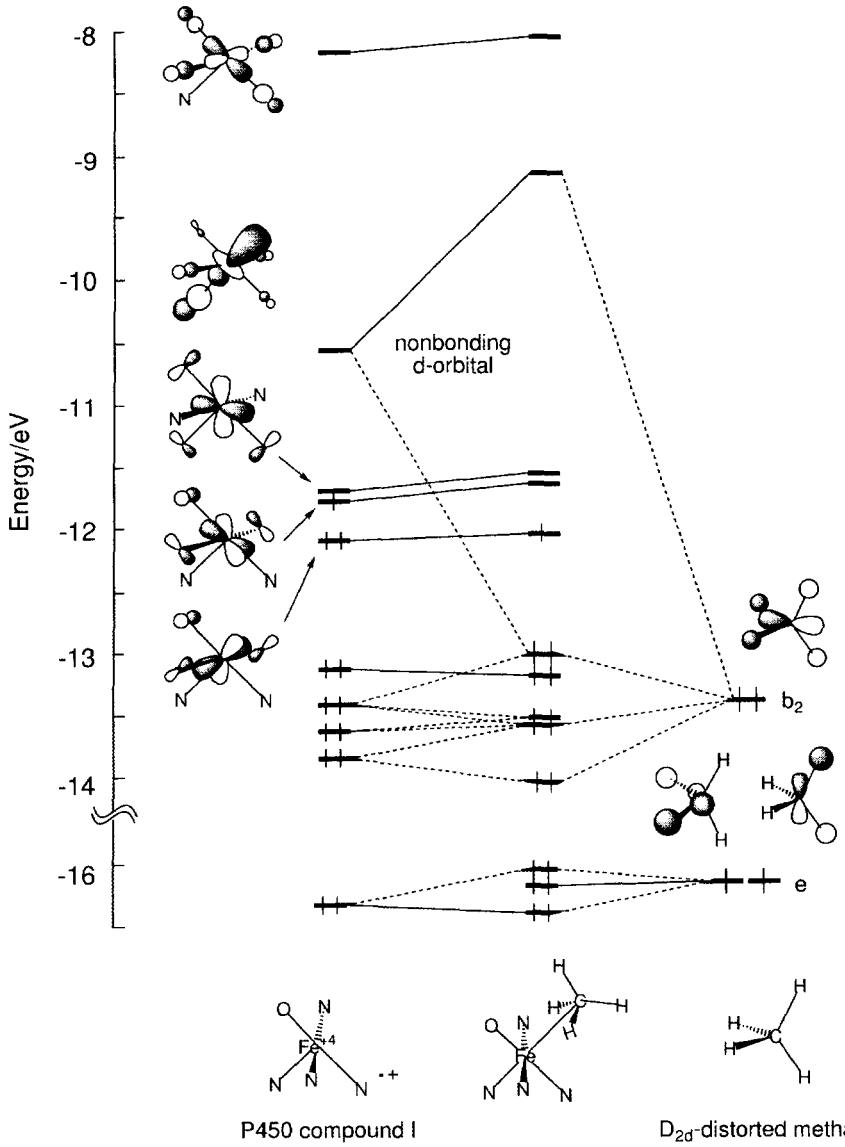
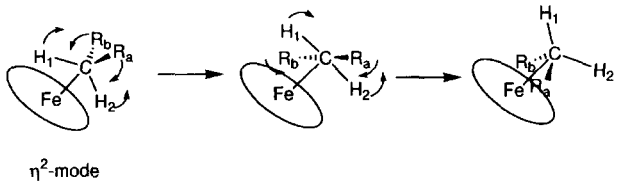


Fig. 3. Orbital interactions between a model active species of cytochrome P450 and a D_{2d}-distorted methane.



Scheme 4.

actant complex significant electron transfer occurs from the methane b_2 HOMO to the unoccupied d orbitals of complex, as indicated in Fig. 3, the activation energy for such an inversion is theoretically expected to be decreased from the value for free methane. In fact, from extended Hückel calculations we obtained activation energies of about 60 kcal mol^{-1} for an inversion at a five-coordinate methane on a model of intermediate **Q** of soluble MMO.[20] The computed values appeared to be significantly decreased from an extended Hückel value of 125 kcal mol^{-1} for the inversion of free methane. We thus think that observed partial inversion of stereochemistry in enzymatic hydroxylations by cytochrome P450 as well as soluble MMO can be ascribed to such a distortion of substrate in a reactant complex. We recently proposed from detailed DFT computations that the inversion of methane can occur on transition-metal complexes under ambient conditions through a thermally accessible transition state.[42] A radical mechanism based on a planar carbon species may not be the sole source of the observed loss of stereochemistry in transition-metal catalyzed hydrocarbon hydroxylations and other related reactions.

Finally we would like to refer to recent mechanistic proposals that seem to be closely related to our model. Collman et al.[43] proposed the reversible formation of an agostic complex between an alkane substrate and a high-valent iron-oxo species during the catalytic hydroxylation of hydrocarbons by P450 models. This is quite a realistic proposal to us. Coordination of an alkane substrate to an Iron(II) porphyrin has been also reported by Evans et al.[44] Moreover, Shaik et al.[45,46] recently suggested from a theoretical point of view

that the adjacency of the high- and the low-spin states of the bare FeO^+ complex has some distinct implications with respect to the C–H bond activation by cytochrome P450. The contexts of these recent papers are in line with the two-step concerted mechanism[11] which was first applied to the methane hydroxylation by soluble MMO[19,21] and cytochrome P450.[47]

Conclusions

On the basis of the two-step concerted mechanism,[12,13] which has been proposed to play a role in the catalytic hydrocarbon hydroxylation by soluble MMO,[21] we discussed the hydroxylation by the oxoferryl active species of cytochrome P450. We proposed that the catalytic reaction is initiated by the formation of a reactant complex involving a five-coordinate carbon species or a σ-complex, followed by concerted hydrogen and alkyl migrations which lead to the formation of a product complex involving alcohol as a ligand, as indicated in Scheme 3. From a natural extension of our mechanistic proposal[21] as well as molecular mechanics and extended Hückel calculations, we predicted that the porphyrin ring is significantly bent both in the reactant complex and in the hydroxy intermediate in order to form an energetically preferred octahedral environment at the iron center. Such bent structures for the porphyrin ring are consistent with observed *cis*-coordination geometries for the M(porphyrin)L_2 complexes. We suggested that possible inversion at the five-coordinate carbon species in the reactant complex should lead to partial inversion of stereochemistry at a labeled carbon. We hope the present proposals will stimulate many new experimental and theoretical studies about the hydrocarbon hydroxylation by cytochrome P450.

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Appendix

Molecular mechanics (MM) calculations were carried out with the Cerius2 program package.[48] The universal force field (UFF)[24,25] we used includes bonded interactions, which consist of bond stretching, bond-angle bending, dihedral-angle torsion, and inversion terms, and nonbonded interactions, which consist of van der Waals and electrostatic terms. Good agreement with experiment is observed when UFF is applied to the conformational equilibria of compounds for which charge apparently does not play an important role. The magnitude of errors in the structures of metal-containing compounds are somewhat larger than those for organic compounds, but comparable to the errors for main group compounds.[25] Experimental metal–C bond distances were reported to be well reproduced by UFF.

We used the YAeHMOP[49] program package in performing extended Hückel calculations. Standard parameters for Fe, C, N, O, and H atoms appear in Table 1, in which H_{ii} is the atomic orbital energy and ζ the Slater exponent. This approximate molecular orbital method models orbital energies, orbital interactions, bond angles, and dihedral angles reasonably well. The geometrical parameters in the reactant complex in Fig. 2 were set from MM calculations. The geometry adopted for the FMO (fragment molecular orbital) analysis in Fig. 3 is as follows: Fe–N = 2.0 Å, Fe–O = 1.7 Å, and Fe–C = 2.2 Å; all bond angles at the iron center are 90°; H–C–H angles of the D_{2d}-type methane are 150°.

Table 1. Extended Hückel Parameters[a] for Fe, O, N, C, and H Atoms

orbital	H_{ii} (eV)	ζ_{i1}	c_1	ζ_{i2}	c_2
Fe 4s	−9.1	1.9			
Fe 4p	−5.32	1.9			
Fe 3d	−12.6	5.35	0.5505	2.00	0.6260
O 2s	−32.3	2.275			
O 2p	−14.8	2.275			
N 2s	−26.0	1.950			
N 2p	−13.4	1.950			
C 2s	−21.4	1.625			
C 2p	−11.4	1.625			
H 1s	−13.6	1.3			

a) H_{ii}, the atomic orbital energy; ζ, the Slater exponent.

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