Quantum chemical studies of methane monooxygenase: comparision with P450

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The catalytic pathways of soluble methane monooxygenase (sMMO) and cytochrome P450CAM, iron-containing enzymes, are described and compared. Recent extensive density functional *ab initio* electronic structure calculations have revealed many similarities in a number of the key catalytic steps, as well as some important differences. A particularly interesting and significant contrast is the role played by the protein in each system. For sMMO, the protein stabilizes various species in the catalytic cycle through a series of carboxylate shifts. This process is adequately described by a relatively compact model of the active site (~100 atoms), providing a reasonable description of the energetics of hydrogen atom abstraction. For P450CAM, in contrast, the inclusion of the full protein is necessary for an accurate description of the hydrogen atom abstraction.

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Abbreviations

cP450	cytochrome P450
DFT	density functional theory
MMOH	methane monooxygenase hydroxylase
QM/MM	quantum mechanics/molecular mechanics
sMMO	soluble methane monooxygenase

Introduction

Conversion of a C–H bond into an alcohol, by formal insertion of an oxygen atom from dioxygen, is a common and important biochemical reaction [1,2]. Activation of the C–H bond at room temperature is a challenging problem, particularly when specificity is desired with regard to reactants and products, as in a biochemical context. A highly reactive oxidant is required, yet this species must perform only the requisite C–H conversion to the alcohol.

To solve this problem, nature has principally relied upon transition-metal-containing enzymes that exhibit complex catalytic cycles. We consider two such enzymes in this review: soluble methane monooxygenase (sMMO) and cytochrome P450 (cP450). sMMO catalyzes the conversion of methane, NADH and dioxygen to methanol and water [3–7,8••,9••,10–12]. A variety of cP450 enzymes have been the subject of experimental investigation [13–18]. Here, we focus specifically on the bacterial enzyme cytochrome P450cam, [19••,20••,21–25] named for its preferred natural substrate, camphor. cP450cam was selected because it is the system for which the greatest amount of kinetic and high-resolution structural data are presently available.

Both the hydroxylase component (MMOH) of sMMO and cP450 employ iron as the transition metal component at the enzyme active site. MMOH is a non-heme diiron protein [5,12,26], whereas cP450 employs a heme iron [27,28] as its catalytic agent. Figure 1 displays the active site structures of the resting forms for both enzymes as determined by X-ray crystallography. Despite considerable differences in these core structures, the transition metal functions similarly in both enzymes, as discussed below.

The catalytic cycles in both MMOH and cP450 are similar, although key details differ significantly. We outline common elements of the catalytic cycle as follows:

1. The resting state is activated by injecting electrons to reduce the iron atom(s) so it (or they) can effectively bind dioxygen. A partner protein capable of long-range electron transfer (MMO reductase [29], and putidaredoxin reductase for cP450 [30–32]) is required.

2. Dioxygen is bound and reduced, displacing a weakly bound water molecule. Reductive activation of dioxygen results in a change of spin and redox state of the iron atom(s) and a substantial conformational change in MMOH.

3. The enzyme converts to form the active species, in which one oxygen atom of the dioxygen is prepared to abstract a hydrogen atom from the substrate.

4. The active species, containing a highly reactive oxygen atom (oxene) attached to one or more Fe(IV) atoms, abstracts a hydrogen from the substrate via a linear C···H···O transition state. Rotation of the resulting –OH moiety created at the catalytic core then allows attack by a substrate carbon leading to the alcohol, which rapidly detaches from the core as the product. This process can proceed either through a bound radical or by a concerted reaction in which this species does not form.

5. The resting state is regenerated.

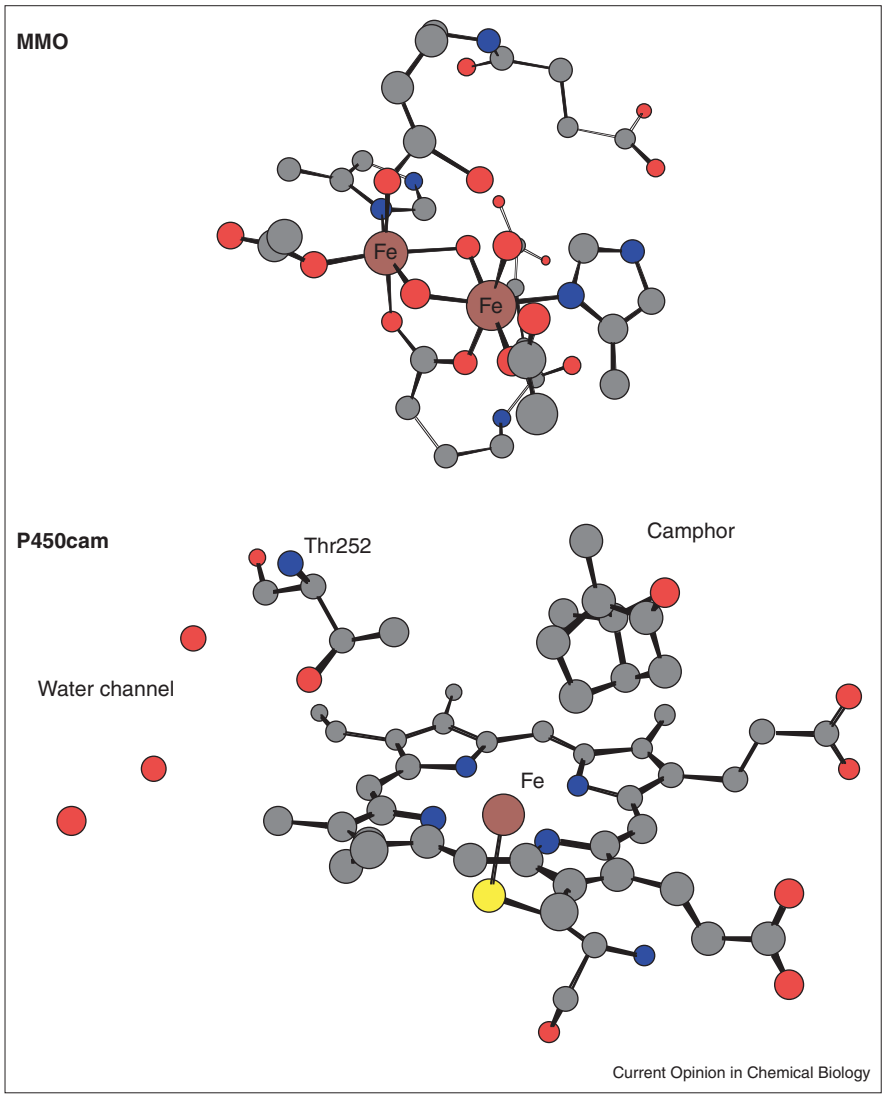
Figure 2 describes these various steps for the MMOH and cP450 catalytic cycles.

Computational methods

Although much has been learned from experimental studies of both MMOH and cP450, a detailed structural and energetic description of the reaction mechanisms is

Figure 1

Geometries from X-ray crystal structures of the core of the active sites of the resting forms of both enzymes.

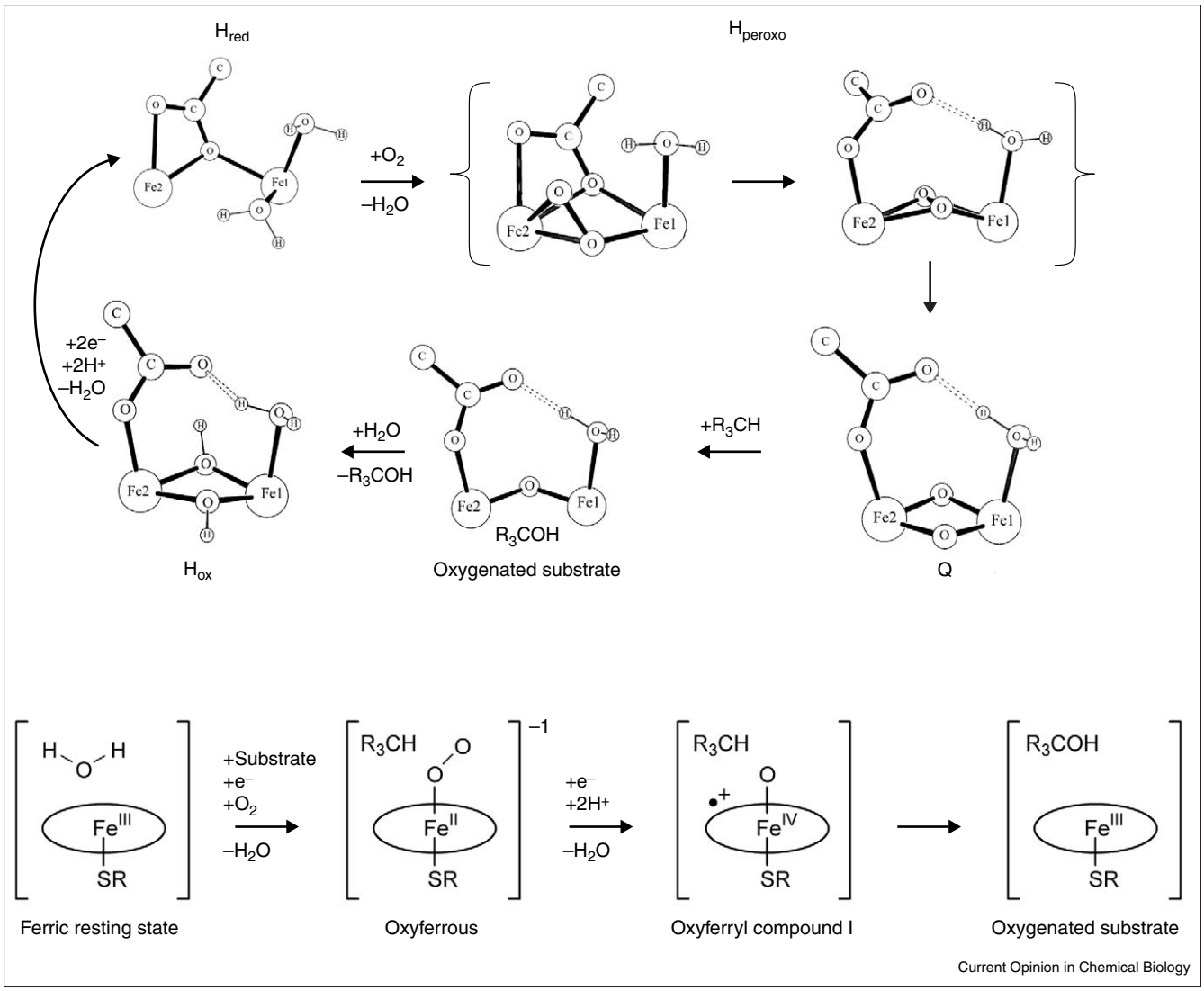


difficult, if not impossible, to formulate, without the use of computational methods. Advances over the past decade in computational chemistry models and algorithms, as well as computer hardware, have rendered tractable the treatment of the reaction chemistry of transition-metal-containing enzymes. The studies reviewed here employ *ab initio* quantum chemical method based on density functional theory (DFT) [33,34], as well as mixed quantum mechanics/molecular mechanics (QM/MM) [35,36] methods in the case of cP450. The Jaguar suite (all quantum chemistry calculations described below were carried out with Jaguar 4.1, Schrödinger, Inc., Portland, OR, 1991–2000) of *ab initio* quantum chemistry programs provide a particularly efficient platform for the treatment of large transition-metal-containing systems, as documented extensively in previous publications [3,4].

DFT methods, when used with large basis sets and a reasonable (if approximate) treatment of open-shell systems, can provide excellent accuracy for relative energetics of transition-metal-containing systems. Using Jaguar, it is possible to include on the order of 150 atoms at a QM level with relatively modest computational effort. QM/MM methods allow a QM region of this size to be embedded in an MM representation of the entire protein. Our expectation is that the energies of species computed by these methods will have an accuracy of ~3–5 kcal mol^{-1}, or better in favorable cases. This degree of accuracy is sufficient for evaluating the relative energies of intermediates and transition states in the cP450 and MMOH catalytic cycles, affording a qualitative understanding of the reaction pathways and allowing one to compare the two enzymes under study.

For MMOH, our results are based on a QM model of ~100 atoms, which contains key protein hydrogen bonds that enforce structural stability at the core region; our model for studying the hydrogen atom abstraction step is shown in Figure 3. The compact, globular nature of the diiron core

Figure 2



Various steps for the MMOH and cP450 catalytic cycles.

makes it possible to obtain meaningful results without including the rest of the protein. For cP450, it is necessary to use QM/MM methods if a quantitative treatment of the catalytic cycle is desired. The heme group, substrate, and key catalytic waters and protein side-chains form a loosely aggregated, delocalized assembly that would disassemble without the protein to hold the various pieces in place. Moreover, the specific geometries at which these components are held are critical for function. The use of QM/MM methods obviates this problem and allows a quantitative assessment of electrostatic stabilization and strain energy imposed by the protein, which is crucial to its catalytic function. The QM region, comprising the groups mentioned above, is ~130 atoms in the studies described below.

For both systems, we employ high-resolution X-ray crystal structures as a starting point for model building and calibration of the methodology. Structures of the reduced and oxidized enzyme have been determined for MMOH by one of our research groups [37,38]; our calculations reproduce these structures to within ~0.5 Å, approaching the experimental resolution. For cP450, we make extensive use of the recently determined structures [19••] that provide the first information about transient intermediates important in that system.

Results

We focus here on steps 3 and 4 of the catalytic process, for which we have produced extensive results that allow the mechanisms for MMOH and cP450 to be contrasted in an interesting fashion. Step 1 is difficult to model quantitatively because of the participation of partner proteins. Experimental structures of the pertinent protein–protein complexes are not yet available, although the MMOH-binding surfaces have been identified for MMOB and

MMOR [39–41]. We have verified that step 2 is thermodynamically favorable in both systems, but quantitative characterization of the processes is still ongoing. Finally, we have not yet investigated step 5.

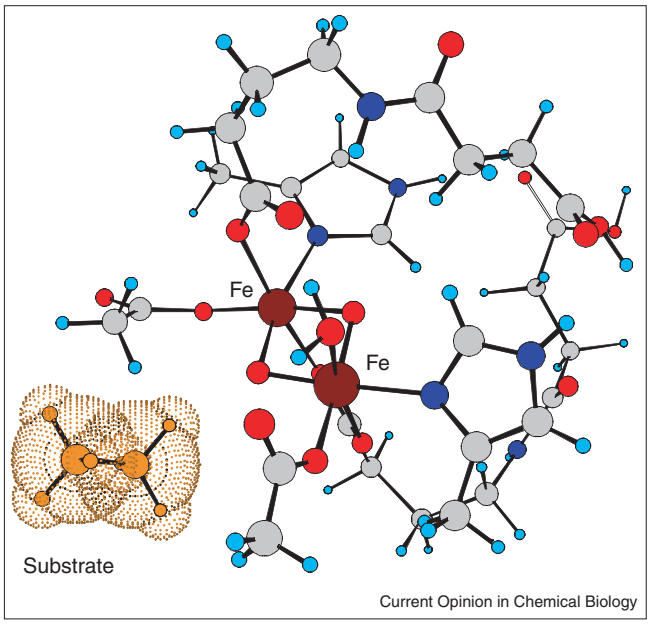
Preparation of the catalytically active species

In MMOH, there are two experimentally observable intermediates, designated H_{peroxo} and Q, the latter being the species competent to convert methane to methanol. In early work [4], we characterized a species generated by the initial addition of dioxygen to the reduced MMOH. We subsequently found [3], however, that it is not possible to convert this structure directly to that of Q; there is a second intermediate, shown in Figure 2, that must be traversed if Q is to be accessed. Its structure is in good agreement with that found independently [42]. As a candidate for the experimentally observed H_{peroxo} intermediate, it has the advantage of symmetrical environments for the iron atoms, which is strongly suggested by its Mössbauer spectrum. We have calculated the transition state in which H_{peroxo} is converted to Q. The enthalpy of activation is 17.95 kcal mol^{-1}, and the reaction is 10.91 kcal mol^{-1} exothermic, which is roughly consistent with the observed lifetime of H_{peroxo}. The structure of Q is shown in Figure 3. The thermodynamic ordering of H_{peroxo} and Q energies, as well as those of the reduced enzyme and the initial intermediate obtained from dioxygen addition, are consistent with the experimental order in which these structures appears.

In cP450, dioxygen is added following addition of the first electron to the substrate-bound enzyme, forming a detectable intermediate. A second electron and two protons are then transferred to the complex to produce a ferryl intermediate and a water molecule, as deduced from kinetic isotope effect measurements [43]. Recent EPR studies [32] and theoretical calculations [44••] have also revealed the need for reduction prior to proton addition. Our calculations demonstrate that the first proton must come from Thr252 through a 3.9 kcal mol^{-1} barrier and in a 3.0 kcal mol^{-1} exothermic process. The threonine proton is replaced, with an almost negligible barrier (1.0 kcal mol^{-1}), by migration of a proton down the water channel evident in Figure 1, which connects the active site with the Asp366 (not depicted). A second proton addition to the distal oxygen cleaves the O–O bond, forming a water molecule that leaves spontaneously to create the ferryl intermediate. Work on this step is currently being undertaken. Our results are consistent with isotopically labeled molecular oxygen studies, which reveal that the proximal oxygen, directly bound to the heme iron, is transferred to substrates, while the distal oxygen is involved in the concurrent formation of water [45,46].

In both enzymes, an oxygen atom is transferred to substrate from a mononuclear Fe(IV) or diiron(IV) center following initial attack on the hydrogen atom of the substrate. The detailed paths to the intermediates are quite different, however. In MMOH, carboxylate shifts and mobile hydrogen bonds are used to insert the O_2 fragment across the diiron

Figure 3



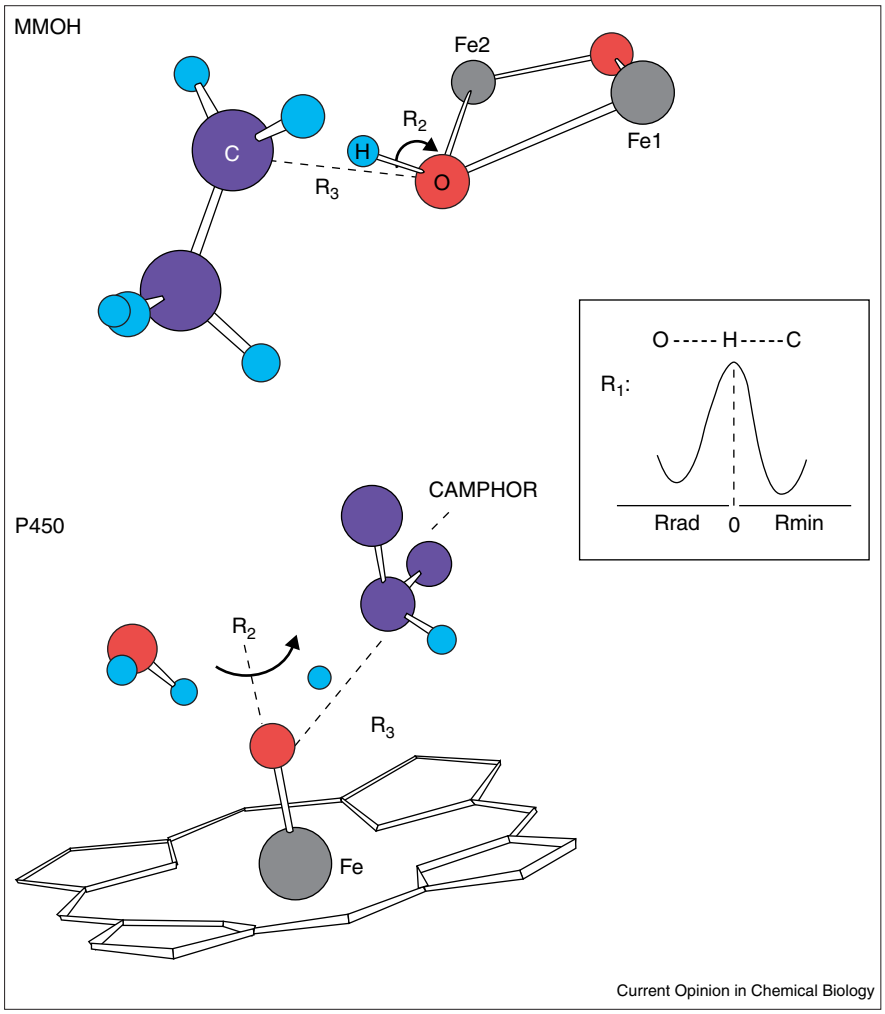
Q intermediate for the MMOH enzyme. The substrate (ethane) is also shown and marked with van der Waals contour.

axis. In cP450, a specifically positioned chain of water molecules (the water channel motif implicated in cP450 activation has already been seen in other P450 enzymes, for example see [47]), terminating in Thr252, selectively deliver protons to dioxygen in conjunction with electron transfer, thus removing one oxygen atom as a water molecule and preparing the second oxygen atom for its C–H bond activation role. Mutation of Thr252 opens the water channel molecules for less selective interaction with dioxygen, possibly introducing a decoupling reaction pathway, in agreement with experimental results [20••]. Computational work investigating this last possibility is currently in progress.

Dynamics of the hydroxylation step

The transition states for both the MMOH and cP450 reactions involve hydrogen atom abstraction, the core geometries of which for both enzymes are depicted in Figure 4. Electronic enthalpies of activation are comparable (16 kcal mol^{-1} in MMO vs. 12 kcal mol^{-1} in cP450). Once the hydrogen is fully transferred to the oxygen atom, hydroxylation is completed by attack of carbon on the bound O–H group, followed by spontaneous release of the resulting alcohol. We have studied the dynamics of this latter stage of hydroxylation in MMOH [9••] by using a multidimensional potential surface generated by extensive DFT calculations, and a semiclassical trajectory methodology that approximately incorporates tunneling and zero-point energy. These calculations indicate that there are two active channels: a radical/recoil rebound channel in which a bound radical intermediate is produced (~85%), and a concerted channel (~15%). In both cases, the –OH group produced by hydrogen transfer must rotate out of the way of the carbon

Figure 4



Main coordinates for MMOH and cP450 hydroxylation reactions. R_1, hydrogen abstraction; R_2, hydrogen rotation; R_3, substrate diffusion.

to enable its attack. The barrier for such a rotation is lower prior to the substrate descending into the radical intermediate well, in which the carbon retreats by ~0.3 Å, hence the presence of the concerted channel. The semiclassical dynamics calculation accurately reproduces the experimental results, predicting the net retention of configuration [48,49] when chiral ethane, CH_3CHDT is used as a substrate [50••]. Although we have not yet carried out a detailed dynamics calculation for cP450, the energetics involved in the hydrogen abstraction, rotation and product formation steps are quite similar to those for MMOH. Therefore, a picture of the hydroxylation reaction similar to that in the latter system is likely to emerge.

Although the mechanisms of hydroxylation in cP450 and MMOH are qualitatively similar, there is a major difference in how the relatively low barrier heights for the reaction are achieved. Understanding and reproducing this feature in model compounds have been exceedingly difficult to accomplish. In MMOH, the non-heme diiron(IV) unit in intermediate Q is intrinsically able to activate the methane C–H bond; such results are reproduced computationally by relatively small models of the active site core. In cP450, however, models in which the ferryl intermediate form of the heme group are considered without the surrounding protein environment fail to duplicate the qualitative energetics for the abstraction reaction. In particular, the relative stabilities of reactant and product predict a ~4 kcal mol^{-1} endothermic reaction, when including the full protein, whereas a ~15 kcal mol^{-1} endothermic energy profile (or higher [51,52]) is computed by reduced *ab initio* models. Similarly, the associated barrier of ~12 kcal mol^{-1} for the QM/MM result is considerably larger for reduced models, ~25 kcal mol^{-1}. Our preliminary analysis reveals that these discrepancies can be addressed by understanding the Fe–S interaction at the site where iron is ligated to the protein by an axial cysteine residue. Geometric constraints by the protein and electrostatic interactions in the region of the sulfur and carboxylate components of the heme group seem to favor the optimum conformation of the product.

Conclusion

Over the past five years, it has become increasingly clear that modern DFT methods, coupled with dramatic reductions

in computational cost/performance, allow metalloprotein enzymes to be modeled quantitatively at an atomic level of detail, with reasonable accuracy for both structures and energetics. Such calculations, when calibrated by experiments, are enabling a qualitatively enhanced understanding of the chemistry that underlies biological function. In the cases of MMOH and cP450, we can delineate features of the intermediates that perform the key C–H bond activation steps. Although there is a great deal more work to be done before the characterization of these systems can be considered to be complete, that objective now appears to be feasible.

Update

Recent work has discussed the role of second oxidant agents for P450s [53]. In addition, Shiro and co-workers [54] found kinetics isotope effects in peroxygenase P450s. Both results support a hydrogen abstraction mechanism with an oxyferryl compound as an active species.

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