Modeling Enzymatic Reactions Involving Transition Metals

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Received January 18, 2006

ABSTRACT
High-accuracy quantum chemistry has now been applied for almost 10 years to biological problems involving transition metal active sites. The leading theoretical method is hybrid density functional theory (DFT), usually with the B3LYP functional. The chemical models vary in size, commonly from 30 to 100 atoms treated fully quantum mechanically. Two schools exist, one using the smallest possible adequate models and the other using as large models as possible and sometimes including the entire enzyme by combining quantum mechanics with molecular mechanics. In our group, we have found that the latter approach, which is much more time-consuming and error prone, is seldom needed. In this Account, methods and models will be described and examples of recent applications given. The examples are chosen to illustrate trends and to show cases where theory has predicted new mechanisms not suggested previously.

1. Introduction

Theoretical enzyme chemistry took a major step forward about 10 years ago when it became possible to attack mechanistic problems involving enzyme active sites containing transition metal centers in a meaningful way.[1] This is a very important part of biochemistry containing enzymes such as photosystem II of photosynthesis, cytochrome *c* oxidase in the respiratory chain, ribonucleotide reductase in DNA synthesis, and methane monooxygenase for converting alkanes to alcohols. Quantum chemical methods have now been used to examine these enzymes and many others, and appropriate methods and models have eventually emerged. In the present Account, leading models will be discussed and examples will be given where theory has provided significant new contributions. The emphasis will be on problems where theory has been shown to be particularly useful. In this context, comparisons of different systems yielding trends is a major point. Another advantage with theory is that suggested mechanisms sometimes relatively easily can be shown to be unlikely and that new suggestions can be made. To prove that a certain mechanism must be correct is a much harder task, both experimentally and theoretically, and will usually require a long combined effort. In this context, theory has risen in importance during the past decade to become a part that needs to be considered before a mechanism can be fully accepted.

2. Methods and Models

The main advantage with quantum chemical calculations is that an energy can be associated with a given structure. From the relation between the energy and the coordinates, equilibrium geometries can be obtained even for very short-lived species and transition states. The respective energies give the thermodynamics and the rate of the reaction studied, provided transition state theory can be applied. The general rule is that transition state theory is sufficiently accurate for barriers of 10 kcal/mol and higher, where the most interesting enzymatic reactions can be found. All simulations of reactions in solutions and enzymes with barriers of 10 kcal/mol or more have given transmission factors close to unity.[2] A typical rate-limiting biochemical reaction occurs on the millisecond to second scale, corresponding to barriers in the range 13−18 kcal/mol. Reactions faster than this have seldom been decisive for the evolution of enzymes.

2.1. Quantum Chemical Methods. The by far leading quantum chemical method in the present context of transition metal biochemistry is hybrid DFT (density functional theory) using the B3LYP functional.[3] It was used in the first study of this kind[1] and is still the first choice in most applications. There are mainly two reasons this method is used. The first one is that B3LYP has been shown to be the most accurate of the DFT functionals in benchmark tests. The second one is that the use of this method is fast enough to be able to treat rather large models, even up to a few hundred atoms.

In its basic form, B3LYP is a density functional, but it also contains Hartree−Fock exchange, which is why it is termed a hybrid DFT functional. The treatment of exchange is, in fact, the major advantage of B3LYP with respect to earlier DFT functionals. The accuracy of the B3LYP functional has been very well tested for molecules containing first- and second-row atoms, where the errors are seldom higher than 3 kcal/mol.[4] For transition metals, there are no benchmark tests, but the accuracy is normally within 3−5 kcal/mol.[5] Recently, a review has been written containing a much more detailed discussion of these general rules.[6] Even though this accuracy is most often sufficient to decide between different mechanisms, an important part of the research in this area is to understand the origin of the errors and to predict when an error is

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10.1021/ar050123u CCC: $33.50
Published on Web 07/13/2006

small or large. The largest errors using B3LYP have been found for the binding energies of ligands such as O_2 and NO to transition metal centers,[7] where the errors in some cases may be larger than 5 kcal/mol. Fortunately, this is seldom the most interesting part of the potential surface. From the point where O_2 (or NO) has become bound and onward, the potential surface is usually quite accurate, including regions of bond breaking and bond formation. Alternatives to the use of B3LYP are the nonhybrid methods (BLYP, BP86, etc.), which have been shown to give accurate geometries, but the energetics is often considerably worse than that for B3LYP.[8]

Ab initio methods such as coupled cluster methods with triples (CCSD(T)) are normally more accurate than B3LYP but require extremely long computation times and can therefore only be applied to small models of at most 20 atoms, and even then with a large computational effort. CASPT2 (complete active space second-order perturbation theory) is able to treat larger systems than CCSD(T) but is somewhat irregular in its behavior, sometimes leading to very large errors, which are only beginning to be understood.[6] Examples where CASPT2 and B3LYP give different ground states have been demonstrated, where CASPT2 sometimes agrees with experiment and B3LYP not.[9,10] However, the errors of B3LYP have not been demonstrated to be larger than the normal 3−5 kcal/mol, which always have to be expected. Several examples of very large errors using B3LYP have also been suggested from CASPT2 studies. However, when the results have been thoroughly analyzed, these examples have instead turned out to indicate large errors of CASPT2. A striking example is the energy difference between the peroxide and bis-μ-oxo structures of copper dimers, which initially appeared to indicate an error of about 40 kcal/mol for B3LYP, since this was the difference from the CASPT2 result.[11] In response to the latter claim, it was argued that B3LYP appeared to give results in better agreement with experiments than CASPT2 did,[12] and quite recently a problem with the way CASPT2 was used in this case was discovered.[13,14] Still, despite these problems with CASPT2, this method remains the most promising ab initio method in this area. It is possible that within the next 5-year period recipes for how CASPT2 should be used can be found, in which case it could be competitive with B3LYP in electronically complicated situations.

2.2. Chemical Models. The accuracy of a quantum chemical calculation depends not only on the computational method used but also on the chemical model chosen. In present day hybrid DFT studies of enzyme mechanisms, there are mainly two different strategies. In one approach, the largest possible model of the enzyme is used, sometimes including the entire enzyme by a QM/MM approach. In contrast, we have advocated a different strategy, where instead the smallest possible, but still reasonably adequate, model of the active site is used.[5] There are two reasons for this. First, calculations on small models are much faster and will therefore allow more careful investigations of different reaction mechanisms. The second reason is at least as important and concerns the reliability of the calculations. A problem that is very difficult to control with a large model is that structures end up in local minima. With a small model, it is generally possible to identify all structural changes in the model during a reaction and make certain that these changes are not artifacts of the model. In QM/MM studies, the only possibility applied so far for transition metal containing enzymes is to go backward and forward along the reaction pathway until there are no more structural changes in the model, a procedure that is tedious and still not free from artifacts. It should be added that interesting possibilities to overcome the multidimensionality problem have been suggested,[15] and semiempirical QM/MM approaches already allow very effective free energy calculations.

In our approach, models as small as possible are used in each case. This means keeping first sphere ligands but avoiding outer sphere ligands that do not form strong hydrogen bonds to a group involved in the reaction. Second sphere charged ligands should in general be kept. Peptide chains and the parts of the amino acids connecting the peptide to the functional groups can in general be avoided. These important reductions of the models have been carefully tested in a number of studies.[16,17] Still the models may not always be small by conventional standards, sometimes up to 150 atoms, but each extension of the model should be carefully motivated. To keep the model reasonably close to the X-ray structure, a few points of the model should be frozen from experiments.[18] This is usually not a critical part of the procedure, and the constraints can normally be released at the final geometries without big effects.[18,19]

There are a large number of cases where extensions of small (30−50 atoms) models have been demonstrated to be unnecessary and hardly change the results.[16,17] In contrast, in a few cases, the use of large (100 atoms) models have appeared to indicate that small models are inadequate. One such case is for methane monooxygenase (MMO).[20] It was concluded that large (100 atoms) models compared to small models in the 60 atoms range will have a nontrivial impact on the quantitative energetics of the catalytic cycle. However, this system was recently reinvestigated with exactly the opposite conclusion.[6] For example, the largest discrepancy of nearly 10 kcal/mol between the large and the small model occurs for the energy difference between the Fe_2(III,III) peroxide (**P**) and the Fe_2(IV,IV) dioxygen cleavage product (**Q**). This difference turned out to be entirely due to an error in the large model calculations. Once this error is corrected, the results are nearly identical. The error was due to a convergence to two different local minima in an unimportant part of the model, which is a severe general drawback of larger models such as QM/MM. Once correct models were used, the other results for MMO are also very similar between the small and large model.

Another one of only a few other cases appearing to show a significant importance of going to a model of 100 atoms was indicated in a study of MMO.[21] It was shown that a binding of a water molecule in the second shell had significant relative effects of about 15 kcal/mol between

two different structures. It was concluded that second shell ligands are indispensable for accurate energetic calculations. It turned out that this water molecule found two different local minima in the two structures, a similar problem as discussed above. One of the minima actually had the water molecule enthalpically unbound to the cluster, which is not reasonable.

The above examples were given to show that there are many other more important aspects of a chemical model than its size. In fact, it is our experience that once the important chemical effects are taken care of, further extensions of the model appear to be one of the least important parts of the modeling, at least for all detailed comparisons performed so far. At the present stage, we find that if the results of a QM/MM model differ significantly from those of its QM part, the most likely explanation is an error in the QM/MM calculation, simply because this type of calculation at present is so much harder to control. The finding that small models are quite adequate not only is of technical interest but also gives important insights into how enzymes work. This information can, for example, be useful when biomimetic model compounds are synthesized. To clarify the above conclusions further, it should be added that once a correct calculation has been performed for a large model, it is in general better than one for a smaller model. However, much more work needs to be done to validate the idea that QM/MM models can consistently and routinely provide improved quantitative accuracy for modeling enzyme reactions. In fact, at the present stage, the situation is rather the opposite.

3. Applications

In this section, we present several examples of applications done in our group. In the first example, the trend in activation barriers for methane hydroxylation is described. It is emphasized that computing a trend for different compounds is very useful for quantifying effects in enzymatic catalysis. The second set of examples were selected to illustrate not only that theory is useful for considering mechanisms already proposed but also that novel mechanistic proposals can be made based on the analysis of computational results. Thus, the mechanisms of methane formation by methyl-coenzyme M reductase (MCR), naphthalene dihydroxylation by naphthalene 1,2-dioxygenase (NDO), side-chain migration in the catalytic reaction of hydroxyphenylpyruvate dioxygenase (HPPD), and ring cleavage by homogentisate dioxygenase (HGD) are discussed below with the emphasis on the new insights provided by the computational studies. Finally, the mechanisms for dioxygen activation by several non-heme iron enzymes are addressed.

3.1. Hydroxylation of Methane. A major advantage with theoretical studies is that trends of reactivities can be studied in detail. This is particularly important for identifying the effects that are most significant for efficient catalysis. Experimentally, potentially important properties of an enzyme active site can usually be found: hydrogen bonds are present, distances are unusual, etc. Some of these properties may be decisive for the catalytic behavior, others not. The factor that decides whether an effect is important is the consequence on the energy. Since an

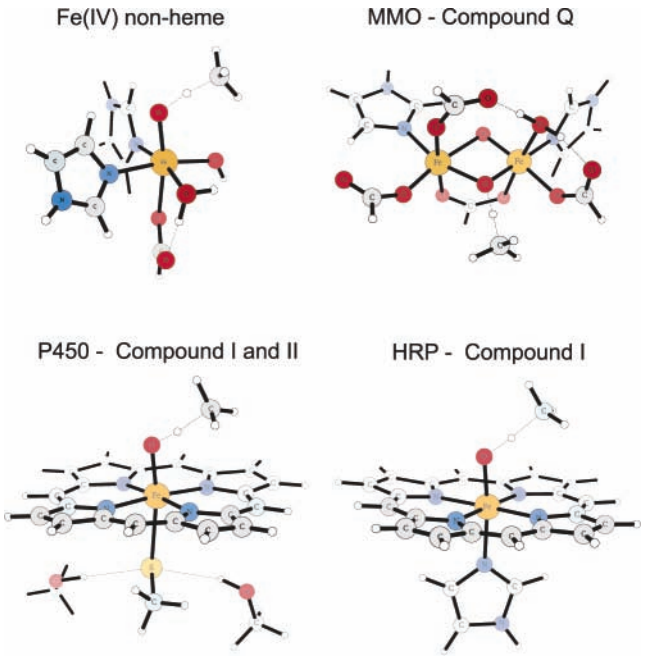

FIGURE 1. The four different types of compounds for which hydroxylation of methane has been compared.

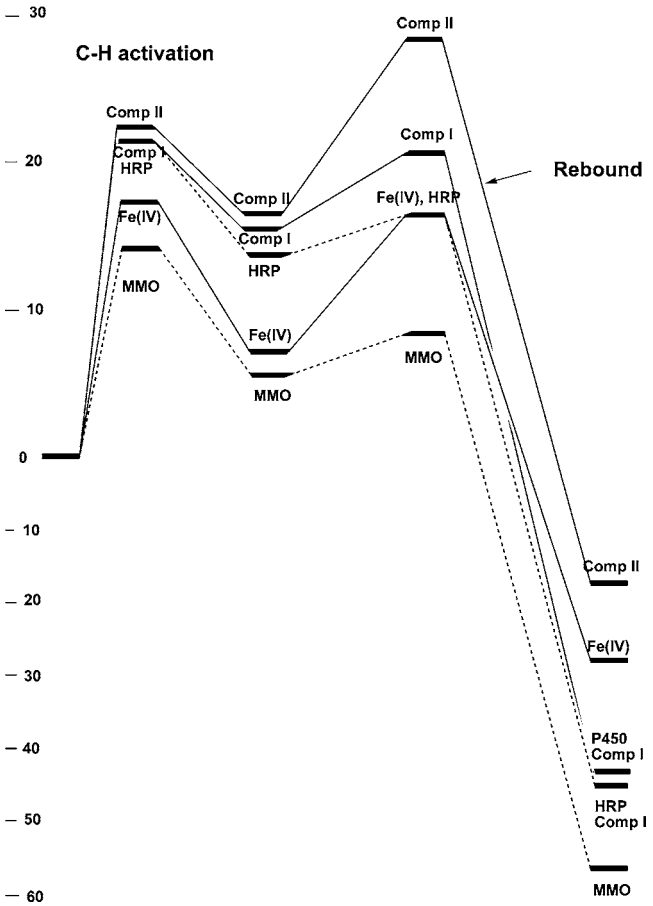

FIGURE 2. Comparison of methane hydroxylation for the different types of compounds shown in Figure 1. Energies in kcal/mol.

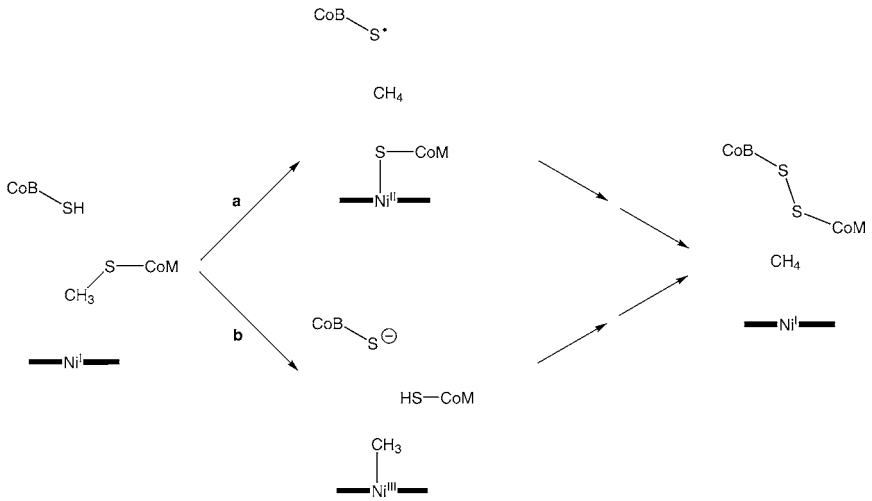

FIGURE 3. Two mechanisms of the reaction catalyzed by MCR: (a) novel mechanism suggested in the theoretical study and (b) previous mechanistic proposal.

energy can be associated with every effect in a theoretical study, it is not seldom found that large distance changes can be relatively unimportant compared to smaller ones, for example.

Enzymatic hydroxylation of methane has been one of the subjects most intensely studied by theory the past decade.[20,22,23] Methane monooxygenases (MMOs) are the only enzymes that can perform this difficult chemical task in nature. There are two forms of MMO, one is soluble s-MMO and one is membrane-bound p-MMO. s-MMO contains an iron dimer at its active site, while the active site of p-MMO is still not clear even though a low-resolution Zn-substituted crystal structure has been determined.[24] The active site of p-MMO has three metal complexes, one with a copper dimer, one with a copper monomer, and one with a zinc monomer. The zinc monomer could in the native enzyme be an iron monomer or perhaps even an iron dimer. Other possibilities are also open. It is in this context interesting to know whether an iron monomer could in principle perform the task of methane hydroxylation. Non-heme iron monomers are known to perform hydroxylations of other hydrocarbons and so are the P450 enzymes. The trend discussed here will thus concern the hydroxylation of methane for an iron dimer like the one in s-MMO, an iron monomer with the common 2His−1carboxylate motif, P450 compounds I and II, and finally horseradish peroxidase (HRP), which has a proximal histidine instead of a cysteine as in P450. These complexes are shown in Figure 1. For P450, two moderately strong hydrogen bonds (from two methanols) have been added since other work has indicated that these bonds could be important.[25]

The first step in the consensus mechanism of methane hydroxylation is a hydrogen atom abstraction from methane by an Fe(IV)−oxo group. The transition states are shown in Figure 1, and the energies are shown diagramatically in Figure 2. Not surprisingly, MMO with two Fe(IV)'s has the lowest barrier with 14.4 kcal/mol. As described in previous studies, the reaction passes through an Fe(V,III) intermediate.[22] However, the Fe(IV) non-heme complex does not have a much higher barrier with only

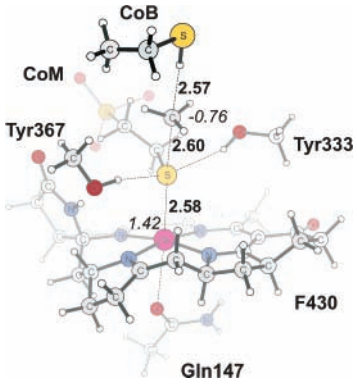

FIGURE 4. Structure of the transition state for the rate-limiting step of the MCR catalytic mechanism. Distances in Å in bold; spin populations in italics.

17.7 kcal/mol. The difference becomes even smaller when entropy effects are included, where the loss of entropy at the C−H activation TS is 2 kcal/mol smaller for the monomer than for the dimer. The conclusion is that a non-heme iron monomer cannot be ruled out as the active species in p-MMO. One problem for the monomer could be that cleaving dioxygen requires four electrons. If the most reduced iron, Fe(II), is the starting state, this means that two external electrons have to be supplied. In p-MMO, the two nearby copper complexes could perhaps supply the additional electrons. If an Fe(II)−O_2H is formed, dioxygen can be cleaved and Fe(IV)−oxo formed with a low barrier.

P450 and HRP have somewhat higher barriers, as expected since they are known not to activate methane, but the difference between them is unexpectedly small. Apparently, the proximal ligand is not so important for this reaction step. The barrier for compound II is not much higher than that for compound I, which is also somewhat surprising. The presence of an additional radical (shared between porphyrin and sulfur) in compound I is of only moderate importance. The effect of the hydrogen bonds in P450 are very small, only 0.3 kcal/mol. On the other hand, the effect of the hydrogen bonds on the sulfur spin is more significant,[25] with a decrease of

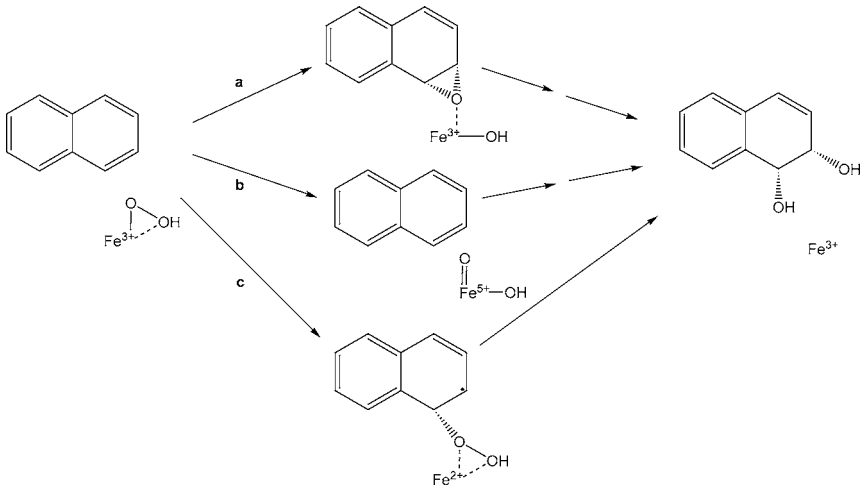

FIGURE 5. Three mechanisms for *cis*-dihydroxylation of naphthalene by NDO: (a) novel mechanism suggested in the theoretical study; (b, c) the two alternative mechanisms.

the spin from −0.80 to −0.51. The reason for the small effect on the energy is the rather small energy involved in the hydrogen bonding. If the hydrogen bonding groups are switched to ammonia, the effects are also very small. The connection between the change of spin on sulfur and the effect on the barrier height is thus quite weak in this case.

For the second step of the hydroxylation mechanism, the rebound step, the differences between the complexes become more pronounced. MMO has a very low barrier with only 2.8 kcal/mol above the methyl radical intermediate, while the corresponding barrier for P450 compound II is 12.2 kcal/mol. This is clearly related to the ease by which the second electron from the substrate can be deposited on the metal. In MMO, an Fe(IV) can take care of the electron, while for P450 compound II, an Fe(III) has to be reduced. This trend increases even more for the final alcohol products.

One result that stands out as particularly striking in the energy diagram is the efficiency of the non-heme Fe(IV) monomer. It is clearly superior to compound I of P450, for example. The origin of this difference is that the non-heme Fe(IV) is high-spin coupled, while the heme Fe(IV) is low-spin coupled.

The above example was chosen to show that trends obtained by DFT calculations can be very useful for illustrating differences between different enzymes and for quantifying the effects responsible for the trends. With access to the energy diagram in Figure 2, the analysis is quite simple. As more results become available, these types of trends will be much more common and should be useful for understanding the chemistry of enzymes and for synthesizing biomimetic complexes.

3.2. Methane Formation by MCR, Methyl-Coenzyme M Reductase. Reduction of simple organic substances to methane is the means by which the methanogenic archaebacteria, which live in anaerobic habitats, acquire the energy necessary for metabolic processes. The final step of methane production is assisted by methyl-coenzyme M reductase (MCR), which catalyzes the formation of a mixed disulfide concomitantly with CH_4 release: CoB−S−H + CH_3−S−CoM → CoB−S−S−CoM + CH_4.[26] Thus, two coenzymes, methyl-coenzyme M (CH_3−S−CoM) and coenzyme B (CoB−S−H), are the substrates of MCR. Interestingly, the active site of MCR hosts a Ni-containing cofactor F_{430}, where the transition metal is chelated by a markedly saturated tetrapyrrole macrocycle. The key point of the earlier mechanistic proposal for MCR (Figure 3b) is the formation of the Ni−CH_3 bond, where the methyl cation is derived from CH_3−S−CoM.[26] However, DFT studies[27] showed that such a Ni−CH_3 bond is far too weak to compensate for the energy required to break the CoM−CH_3 bond (the calculated endothermicity for the first step of such a mechanism exceeds 45 kcal/mol), which renders this mechanism unlikely. Instead, a new mechanism was proposed for MCR (Figure 3a), where in the rate-limiting step involving a barrier of 19.5 kcal/mol, the Ni−S−CoM bond is formed and a methyl radical is liberated (Figure 4).[27] This reactive radical species abstracts a hydrogen atom from CoB−SH with no additional barrier and forms methane. From the calculations performed for models of various sizes, the individual contributions to the barrier height for this mechanism were estimated. Most importantly, the Ni−S−CoM bond strength (38.6 kcal/mol) is remarkably larger than the calculated value for Ni−CH_3 (at most 25 kcal/mol), which is the major factor contribut-

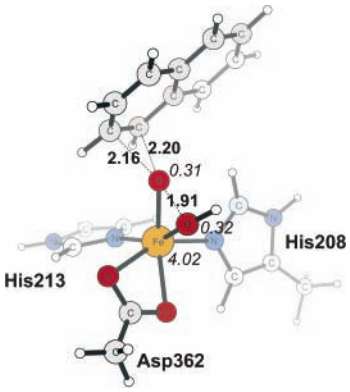

FIGURE 6. Structure of the transition state for the rate-limiting step of the NDO-catalyzed *cis*-dihydroxylation of naphthalene. Distances in Å in bold; spin populations in italics.

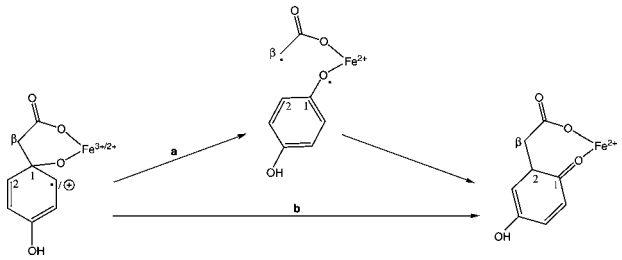

FIGURE 7. Two mechanisms of the side-chain migration in the catalytic reaction of HPPD: (a) novel mechanism suggested in the theoretical study and (b) previous mechanistic proposal.

ing to the lowering of the activation barrier for the suggested mechanism (Figure 3a) with respect to the earlier mechanistic proposal (Figure 3b). (It should be added that this energy difference does not significantly change with other functionals even though the absolute values change by nearly 10 kcal/mol.) In addition, the presence of hydrogen bonds between Tyr333, Tyr367, and the thiol group of CoM (Figure 4) lowers the barrier by 5.5 kcal/mol.[27]

3.3. *cis*-Dihydroxylation by NDO, Naphthalene 1,2-Dioxygenase. Catabolism of naphthalene in bacteria is initiated by naphthalene 1,2-dioxygenase (NDO), which catalyzes the dioxygenation of naphthalene to *cis*-dihydrodiol (Figure 5). The available X-ray structure of NDO and the results of several experimental studies indicate that the species responsible for naphthalene dioxygenation is the mononuclear non-heme iron complex, in which a ferric ion binds a hydroperoxo anion in a side-on fashion.[28,29] There are three possible reaction mechanisms connecting the reactant and product complexes (Figure 5). Thus, in mechanism c the hydroperoxo radical attacks the naphthalene ring forming an Fe(II) intermediate, which through O−O bond cleavage coupled to the second C−O bond formation decays into the diol product. In mechanisms b and a, the hydroperoxo anion acts as an electrophile performing a two-electron oxidation of either iron or naphthalene. Thus, the first step would lead to the Fe(V)−oxo, found feasible in a biomimetic system,[30] or an arene−oxide intermediate for mechanism b and a, respectively. In a DFT study, these plausible mechanisms were studied, and based on the calculated energetics, mechanism a was argued to be the most likely one used by NDO.[31,32] Prior to this theoretical study, this particular mechanism had not been considered for NDO. The calculated energy barriers for all three mechanisms (Figure 5) are 17.5, 26.5, and 25.1 kcal/mol for a, b, and c, respectively. From the optimized structure of the transition state for the rate-limiting epoxidation of naphthalene (Figure 6), it can be seen that indeed the epoxide formation takes place concertedly with O−O bond cleavage, while the spin population on iron shows that the metal does not change its oxidation state at this step and remains Fe(III).

3.4. Side-Chain Migration by HPPD, Hydroxyphenylpyruvate Dioxygenase. The catalytic reaction of hydroxyphenylpyruvate dioxygenase (HPPD) involves unprecedented 100% efficient 1,2 shift of the carboxymethyl

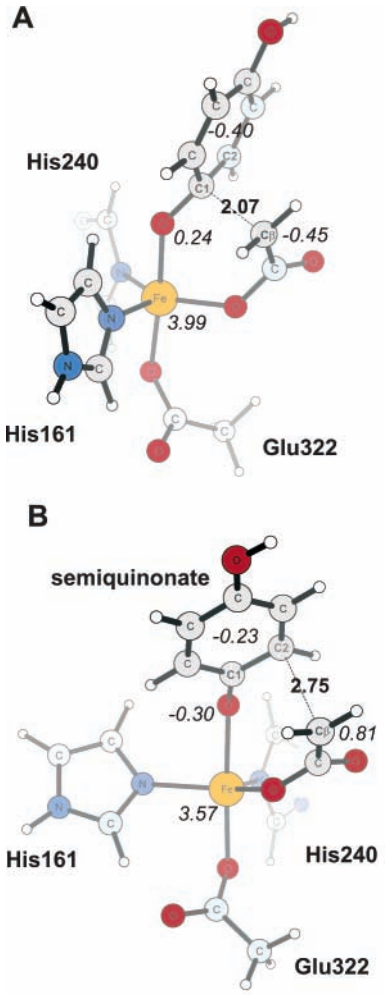

FIGURE 8. Structures of the two transition states for the side-chain migration in the catalytic reaction of HPPD: (A) TS for C1−Cβ bond cleavage; (B) TS for C2−Cβ bond formation. Distances in Å in bold; spin populations in italics.

ring substituent (Figure 7).[33] Similar 1,2 rearrangements, common among aromatic ring oxidizing enzymes, usually involve hydrogen atom migration and proceed with efficiency lower than 100%. These are known as NIH shifts. The mechanism of the HPPD-catalyzed rearrangement was originally suggested to parallel that accepted for NIH hydride shift; that is, a single-step migration of the side chain in the σ-cation,[34] which is produced in the electrophilic attack of the Fe(IV)−oxo species on the aromatic ring (Figure 7b).[35] However, the computational DFT study showed that the carboxymethyl side-chain shift is a two-step process (Figure 7a).[36] Thus, in the first step, the C1−Cβ bond is homolytically cleaved, which leads to a semiquinonate intermediate and carboxymethyl radical coordinated to Fe(II). In the second step, these two radicals recombine through a C2−Cβ bond formation, which leads to the keto form of the product, homogentisate. From the structures of the two transition states for C1−Cβ cleavage and C2−Cβ bond formation (Figure 8), it is easy to recognize that indeed the cleavage and the formation of the two C−C bonds are separate events. The initial C1−Cβ bond cleavage is the rate-limiting process

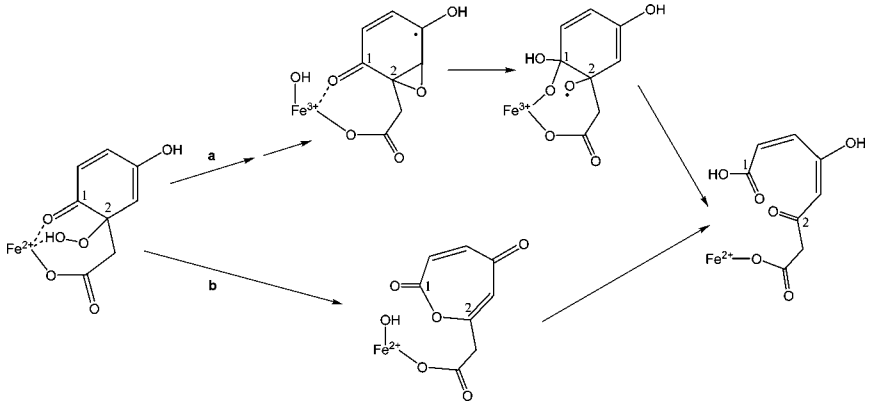

FIGURE 9. Two mechanisms for ring cleavage in the catalytic reaction of HGD: (a) novel mechanism suggested in the theoretical study and (b) previous mechanistic proposal.

involving a barrier of 17.7 kcal/mol, while the calculated activation energy for the rebound step is only 2.6 kcal/mol.

3.5. Ring Cleavage by HGD, Homogentisate Dioxygenase. Homogentisate dioxygenase (HGD) is an Fe(II)-dependent enzyme catalyzing oxidative ring cleavage in the catabolism of tyrosine and phenylalanine. The key step of this process is the transformation of the bridging hydroperoxo structure into the ring-opened product, maleylacetoacetate (Figure 9). The mechanism originally proposed for this step (Figure 9, path b)[37] involves a Criegee rearrangement of the organic hydroperoxide, where the leaving OH anion is supported by Fe(II), while the other oxygen atom is inserted into the C1−C2 bond of the ring. The lactone intermediate produced in this step is hydrolyzed by the Fe-bound hydroxyl group. However, a DFT study indicates that the ring-cleavage step is a radical process involving the formation of an arene oxide radical/Fe(III)−OH intermediate (Figure 9, path a); that is, a novel mechanism was proposed.[38] Thus, the peroxide is cleaved homolytically with the help of the iron ion, instead of acting as an electrophile in the attack on the ring. The calculated barriers for the formation and decay of the arene oxide radical intermediate (path a) are 10.7 and 12.3 kcal/mol, respectively, while the barrier for Criegee rearrangement (path b) is 23.0 kcal/mol. The optimized TS for the final ring cleavage step is presented in Figure 10. The distinctive feature of this mechanism is the fact that the ring is cleaved once the two adjacent carbons bind all oxygen atoms necessary for the formation of the proper product, which leads to a ring highly activated for C−C bond cleavage and an early character of the corresponding TS (Figure 10).

3.6. Dioxygen Activation by Mononuclear Non-Heme Iron Enzymes. Over the past seven years, several different classes of mononuclear non-heme iron-dependent oxygenases have been studied in our group.[32] The mechanisms utilized by these enzymes for dioxygen activation are compared in Figure 11, which should aid the reader to follow the discussion of similarities and differences in the mechanistic strategies used by these enzymes. The

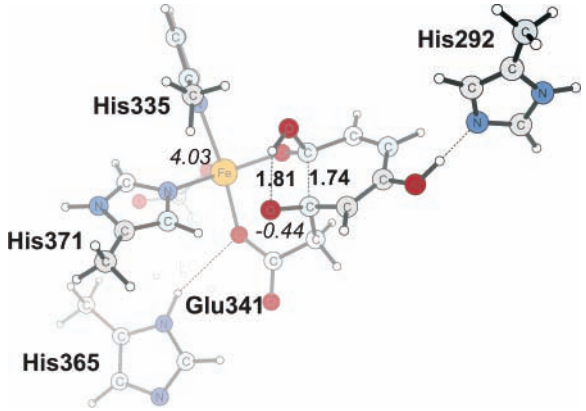

FIGURE 10. Structure of the transition state for the ring-cleavage step in the catalytic reaction of HGD. Distances in Å in bold; spin populations in italics.

general common feature of all of these mechanisms is the presence of the peroxo intermediate, which is the second structure from the left in the mechanisms sketched in Figure 11. Two electrons, necessary for reduction of dioxygen into these peroxo intermediates, are usually supplied by the (co)substrate.[40−43,46] The exceptions are isopenicillin N synthetase (IPNS),[39] 1-amino-1-cyclopropane carboxylic acid oxidase (ACCO),[44] and Rieske dioxygenases,[45] where one electron is supplied either from an external source (ACCO and Rieske dioxygenases) or from the active-site iron (IPNS). The second general theme is the "leaving group support" during the O−O bond cleavage step in the productive decay of the peroxo intermediates. More specifically, the detailed analysis of the structures of the products of the O−O bond cleavage (Figure 11) reveals that the two oxygen atoms derived from dioxygen form in total four covalent bonds, two per atom. Thus, for an efficient O−O bond cleavage mechanism, a proton is usually necessary for stabilization of the leaving OH group. The exceptions are α-ketoacid-dependent dioxygenases, where during the cleavage of the peroxoacid intermediate, the "leaving" oxygen atom develops a second covalent bond with the adjacent carbon. Concerning the mechanism of O−O bond cleavage, it is interesting to note that in most cases it is in total a heterolytic process, that is, it is a two-electron reduction of the peroxo

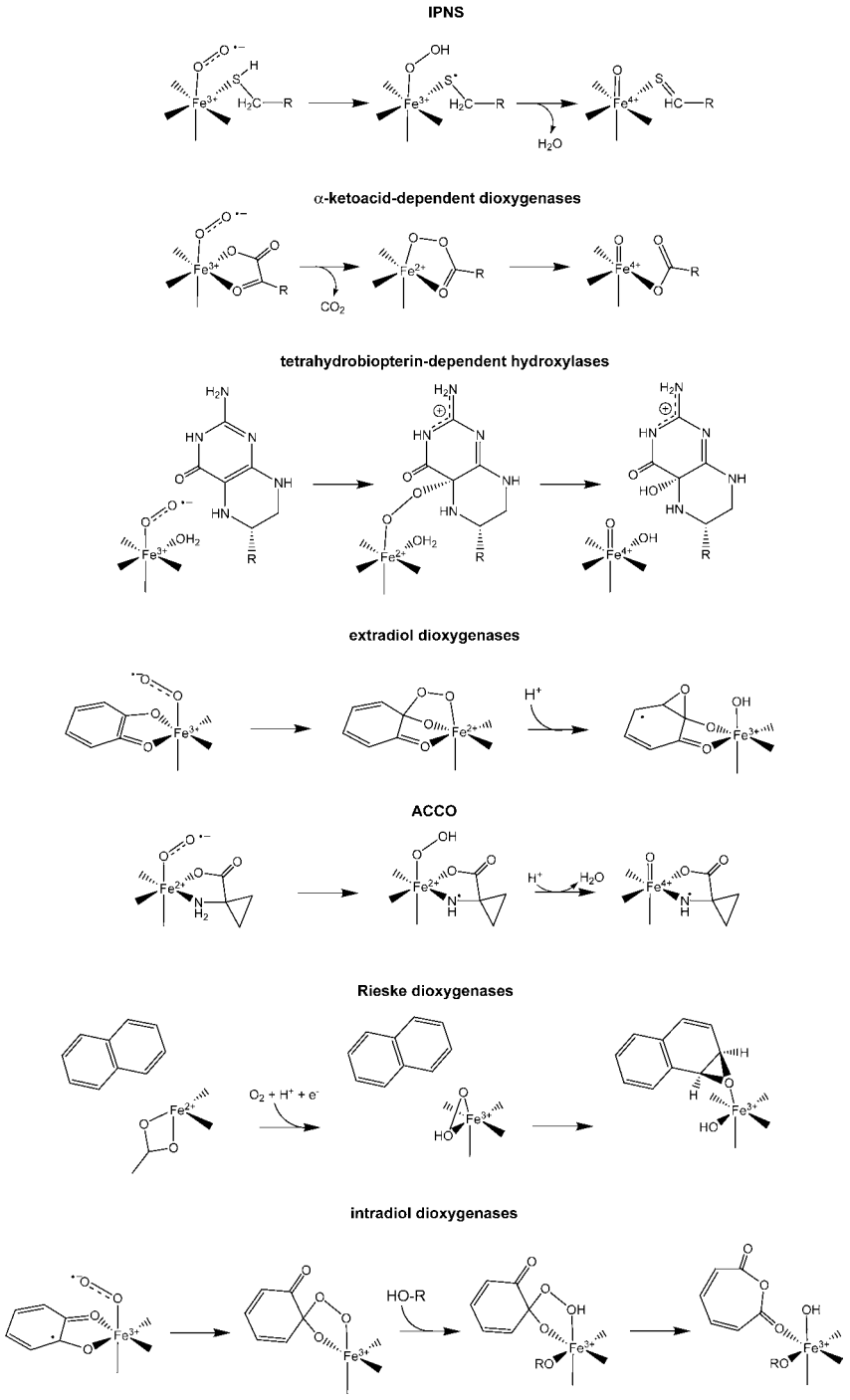

FIGURE 11. Dioxygen activation by mononuclear non-heme iron enzymes.

group. However, it is often found to consist of two one-electron steps, where the transfer of the first electron to the peroxo group is rate-limiting while the second step usually involves a low activation barrier. The only exceptions are extradiol dioxygenases, where the homolytic O−O bond cleavage leads to the arene−oxide radical intermediate.[38,43] The source of these two electrons differs from case to case. Thus, in IPNS, one electron is provided by iron, while the second comes from the substrate radical.[39] In α-ketoacid-dependent dioxygenases,[40,41] tetrahydrobiopterin-dependent hydroxylases,[42] and ACCO,[44] the ferrous ion provides both of these electrons, which leads to the formation of the Fe(IV)−oxo intermediate. Finally, in Rieske dioxygenases[45] and intradiol dioxygenases,[46] where the peroxo group is bound to the ferric ion, the two electrons are supplied by the organic substrate. Thus, the emerging picture is that the Fe(II)-binding peroxo intermediates in mononuclear non-heme iron enzymes may decay either in a homolytic or in a heterolytic process with one or two electrons provided by the metal ion, while the Fe(III)-binding species decay in a heterolytic process, where the ferric ion is not redox active and both the necessary electrons come from the organic substrate. Future studies on other mononuclear non-heme

iron-dependent enzymes will show whether this general picture holds.

4. Conclusions

Examples of results of quantum chemical model calculations on transition metal containing enzymes have been described. These results were obtained with hybrid DFT with the B3LYP functional. The chemical models were designed to cover the main chemical effects, not more. This means that in the work described above uncharged second shell ligands, peptide backbones, and links between the peptide and the functional groups of the amino acids could be left out without loss of accuracy. In this way, models that are unproblematic in terms of local minima and efficient for testing different mechanisms have been obtained. The presence of local minima have been shown to cause severe problems for transition metal catalyzed reactions when very large models are used. At present, there is no practical solution for safely avoiding this problem with large models, but this remains a major task for future method development in this area. The very good performance of small models not only is of technical importance but also is important for understanding the main effects of enzyme mechanisms. It should perhaps be added that the conclusion that small models are very good concerns the variety of reactions described above but is not necessarily generally true. For example, good absolute pK_a values and redox potentials cannot be obtained by small models without an empircial procedure,[17] and a QM/MM calculation is therefore probably better. Another example, taken from MMO, is the description of the weak binding between the metals in their reduced states, where they are connected by extended hydrogen bonding networks.[20] For a small model, this would have to be described by freezing some atoms at their experimental positions, which is probably more arbitrary.

A powerful way of quantitatively understanding the different effects appearing in a particular mechanism is to study trends of similar reactions in different enzymes. An example was given where methane hydroxylation was studied for four different classes of enzymes. The most interesting result is that non-heme iron monomers can catalyze this reaction very efficiently, almost as well as MMO does but not quite. Another example of where studies of many enzymes help in understanding a complicated mechanism is for dioxygen activation. A large number of different cases have been studied using the same methodology and same type of chemical models. The most important conclusion from these investigations is that in the active sites binding ferrous ion the peroxo species can be cleaved homo- or heterolytically, while for the ferric species the cleavage is exclusively heterolytic with two electrons provided by the organic molecule. A common and useful way to use theory is to test whether a suggested mechanism is feasible. This is probably the situation where theory will find its most use in future applications. However, some examples were described here where theory was also used to predict novel mechanisms for important enzymes.

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AR050123U