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Activation of the C−H Bond of Methane by Intermediate Q of Methane Monooxygenase: A Theoretical Study

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The hydroxylase component (MMOH) of the multicomponent soluble methane monooxygenase (MMO) system catalyzes the oxidation of methane by dioxygen to form methanol and water at non-heme, dinuclear iron active sites. The catalytic cycle of MMOH is well established.[1,2] Following reduction of the Fe(III)-Fe(III) resting enzyme to the Fe(II)Fe(II) state, two spectroscopically observable intermediates, H_{peroxo} and Q, are formed upon reaction with dioxygen. Q is a high-valent Fe(IV)Fe(IV) species that oxidizes methane.[3,4] Previously, we described the construction of a large-scale theoretical model (~100 atoms) of the MMOH active site that energetically and structurally reproduced the species in the catalytic cycle.[5] In the present paper, we use ab initio quantum chemical density functional (DFT) methods employing the B3LYP functional[6,7] to examine the reaction by which intermediate Q converts methane to methanol. Computational methods and structural modeling strategies closely follow those of ref 5; computational details of the present work (e.g. basis sets) are given in Table S1.

The theoretical structure determined previously for Q (Figure S1) resembles others in the literature in having a di-μ-oxo core with both iron atoms in the Fe(IV) oxidation state.[3,8,9] Our Q structure differs from others in the literature, however, in that the water molecule bound to Fe1 is not displaced by Glu243,[8,9] yielding a much lower energy than the alternatives. While modeling the reaction of Q with methane, we identified another structure for this intermediate that is 8.8 kcal/mol lower in energy than that reported previously.[5] The new alternative Q structure, depicted in Figure 1, is nearly identical to the previous one except that the water molecule on Fe1 is hydrogen bonded to the other oxygen atom of Glu243. The new structure has key properties, including spin, charge, and Fe−Fe distance, that are comparable to those of the previous structure. Our investigation of the methane reaction reveals little difference when using either structure; below, we employ the structure in Figure 1 because it is lower in energy.

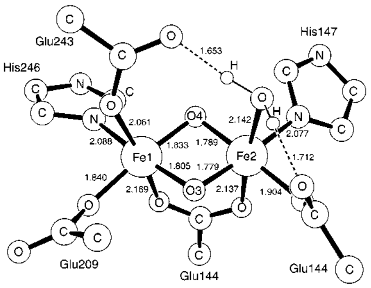

Figure 1. Core structure of the minimized lower energy, isomeric Q model. Numbers indicate bond distances (Å) and dashed lines indicate hydrogen bonding interactions. The Fe−Fe distance is 2.700 Å.

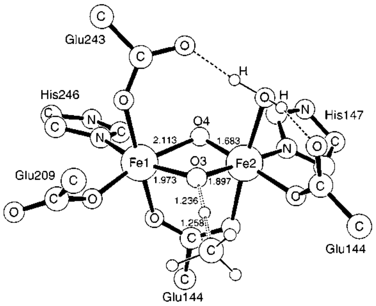

Figure 2. Core structure of the minimized transition state resulting from hydrogen atom abstraction. The numbers indicate distances (Å) from the Fe atoms to the μ-oxo atoms, and from the carbon atom of methane and one μ-oxo atom to the “shared” hydrogen atom. The Fe−Fe distance is 2.979 Å. Dashed lines represent hydrogen bonds.

The calculations began by first docking methane into the complex to form a transition state that would lead to methanol product. In contrast to earlier calculations,[9] we considered only an approach to the core via a path opposite the two histidine residues, since a hydrophobic substrate binding cavity exists at this location in the full protein structure.[10] After many alternatives failed to provide an energetically reasonable result, we concluded, as in refs 8 and 9, that the only way in which a reaction can take place is for methane to approach a bridging oxo atom in the core straight on. We rule out any possibility of direct involvement of the iron atoms in the catalytic process. The transition state is depicted in Figure 2. Our calculations predict an activation energy of 13.2 kcal/mole for the reaction, including an estimated zero point correction of 4.8 kcal/mole computed in ref 11.

Over the past several years, a considerable amount of data have been accumulated concerning the kinetics of the methane reaction.[2,4] A key issue, raised by the structure in Figure 2 as well as the experimental data and in previous theoretical modeling of MMO,[8,9,11] is whether a radical rebound or a concerted mechanism is operative. Experiments with radical clock substrate probes employing MMOH from the *M. capsulatus* (Bath) system suggest that a radical rebound mechanism is not feasible, since the lifetime of the radical would have to be shorter than ~150 fs to be consistent with the failure to observe radical-derived ring-opened products.[12,13] Alternative experiments with ethane made chiral by virtue of the three isotopes of hydrogen yield a retention of configuration of ~70%.[14,15] Although the majority product argues

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(1) Liu, K. E.; Lippard, S. J. *Adv. Inorg. Chem.* **1995**, *42*, 263−289.

(2) Wallar, B. J.; Lipscomb, J. D. *Chem. Rev.* **1996**, *96*, 2625−2657.

(3) Shu, L.; Nesheim, J. C.; Kauffmann, K.; Münck, E.; Lipscomb, J. D.; Que, L., Jr. *Science* **1997**, *275*, 515−517.

(4) Valentine, A. M.; Stahl, S. S.; Lippard, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 3876−3887.

(5) Dunietz, B. D.; Beachy, M. D.; Cao, Y.; Whittington, D. A.; Lippard, S. J.; Friesner, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 2828−2839.

(6) Johnson, B. G.; Gill, P. M. W.; Pople, J. A. *J. Chem. Phys.* **1993**, *98*, 5612−5626.

(7) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372−1377.

(8) Siegbahn, P. E. M. *Inorg. Chem.* **1999**, *38*, 2880−2889.

(9) Basch, H.; Mogi, K.; Musaev, D. G.; Morokuma, K. *J. Am. Chem. Soc.* **1999**, *121*, 7249−7256.

(10) Rosenzweig, A. C.; Frederick, C. A.; Lippard, S. J.; Nordlund, P. *Nature* **1993**, *366*, 537−543.

(11) Siegbahn, P. E. M.; Crabtree, R. H. *J. Am. Chem. Soc.* **1997**, *119*, 3103−3113.

(12) Liu, K. E.; Johnson, C. C.; Newcomb, M.; Lippard, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 939−947.

(13) Choi, S.-Y.; Eaton, P. E.; Kopp, D. A.; Lippard, S. J.; Newcomb, M.; Shen, R. *J. Am. Chem. Soc.* **1999**, *121*, 12198−12199.

(14) Priestley, N. D.; Floss, H. G.; Froland, W. A.; Lipscomb, J. D.; Williams, P. G.; Morimoto, H. *J. Am. Chem. Soc.* **1992**, *114*, 7561−7562.

(15) Valentine, A. M.; Wilkinson, B.; Liu, K. E.; Komar-Panicucci, S.; Priestly, N. D.; Williams, P. G.; Morimoto, H.; Floss, H. G.; Lippard, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 1818−1827.

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against a radical rebound mechanism, the results do not mandate a completely concerted reaction since the stereochemistry in ~30% of the products is lost. In addition, experiments with the radical clock 1,1-dimethylcyclopropane in the MMO system from *M. trichosporium* OB3b suggest that a radical-type mechanism is operative some of the time.[16]

Our first step in understanding the reaction mechanism was to search for a radical intermediate. We did locate a radical intermediate state, the core of which is shown in Figure S2, in which the carbon moves 0.44 Å back from, and the H moves toward, the oxo bridge, yielding a standard O−H bond. This structure is similar to that determined by Morokuma and coworkers.[9] As in that work, methanol formation can most easily proceed from the radical intermediate by rotation of the O−H group to make a 93° angle with the C−O axis, whereupon the carbon will spontaneously move toward the O atom to form methanol bound to the diiron core. This molecule can then leave the core in a barrierless fashion, as discussed below. The barrier to rotation of the O−H group to an appropriate position, computed by us to be 3.9 kcal/mol, is significantly lower than the 6.8 kcal/mol obtained in ref 9, but it is still too large to explain the chiral ethane results. The time scale for the rotation is estimated to be ~5−10 ps, sufficient for the ethyl radical to racemize completely. The energies for these states are summarized in Table S2(b).

There is another path that the system can take from the transition state in Figure 2, however, that affords methanol with a negligible barrier. The first key observation is that, if the system can reach a geometry (by whatever means) in which the H−O−C angle is ~80−90° and the O−H distance is ~0.97 Å (the O−H bond length), methanol forms with no barrier. This finding led us to inquire whether there is a direct barrierless (or nearly barrierless) path from the transition state to that point in phase space. We have located such a path having the following characteristics. Starting from the structure in Figure 2, in the first step the hydrogen forms an O−H bond of 0.97 Å with minimal movement of other atoms. The carbon atom does not recoil, as in the radical path described above. This process has no barrier and is 2.6 kcal/mol downhill.

The next step is for the hydrogen to rotate out to the 80−90° angle described above. Rotation of the H in the opposite direction is very high in energy, as is rotation prior to formation of the O−H bond; thus, there is only one low-energy path to products. As the H rotates, the carbon also moves, rotating in the opposite direction. Table S2(a) presents a series of states in which the H is rotated toward 90°; the C−O distance and angle of the C with the Fe−O−Fe plane are also given, as well as the total energy. The key elements of the reactive core for each of these states are depicted in Figure S3. The transition state for the process (Figure 3) is at a hydrogen rotation angle of approximately 40° and the barrier to the hydrogen rotation is on the order of 1.0 kcal/mol.

Note that the pathway we have described connects a metastable state (the transition state) with the products and hence the highest point on the pathway cannot be located easily by the usual transition state finding algorithms; instead, we have characterized the potential surface by restrained geometry optimizations. The pathway will compete against steepest descent to a radical intermediate, however, because many of the substrate molecules upon reaching the transition state will either have enough kinetic energy to cross the small (1 kcal/mol) barrier on the pathway to products or will tunnel through that barrier to reach products. On the basis of such a picture, it is feasible that this channel will proceed at a rate leading to substantial retention of configuration as required by the chiral ethane experiment, and with the velocity implied by the radical clock experiments.

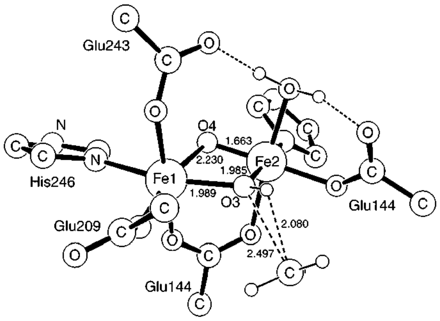

Figure 3. Core structure of the minimized transition state in the concerted reaction pathway. The numbers indicate distances (Å) from the Fe atoms to the μ-oxo atoms and from the carbon atom of methane and one μ-oxo atom to the "shared" hydrogen atom. The Fe−Fe distance is 3.041 Å. Dashed lines represent hydrogen bonds.

We have also analyzed the distribution of unpaired spins for the reactant, transition state, radical intermediate, and various geometries along the concerted reaction and radical intermediate pathways; this information is presented in Table S3. In the reactant each iron atom has $S = 2$ and the two are coupled antiferromagnetically. In the transition state the methyl carbon has a spin population of ~0.5; this value increases to near unity in the radical intermediate and for the early stages of the concerted pathway, with a decay to zero as methanol is formed.

Computation of the partitioning ratio of the two pathways and concomitant prediction of experimental results requires an explicit dynamics calculation on the potential energy surface of the reaction. Here, we simply propose the hypothesis that the chiral ethane results can be explained by the presence of both a radical rebound and a concerted channel (see Figure S5), with partitioning consistent with the experimental data for retention of configuration. For the radical clock experiments, the radical rebound channel is less favorable energetically and/or kinetically, since the larger, heavier molecules might have more trouble reversing trajectory to move backward once the transition state is reached. Thus, the radical contribution to the reactive pathway might be too small to be observed given the detection limits of the experiment.

As mentioned above, an energy minimization protocol revealed that, once the hydrogen rotates out of the plane and a C−O bond is formed, the resulting methanol moiety spontaneously leaves the diiron core in a barrierless fashion. Interestingly, the Fe−O bonds remain essentially symmetric during such optimization. Table S2(c) presents energies and Fe−O bond lengths for a series of structures along the trajectory, and Figure S4 depicts the final stage of this trajectory where the O atom of methanol is bound only loosely in a bridging position between the iron atoms.

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Supporting Information Available: Tables S1−S3 and Figures S1−S5 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[16] Ruzicka, F.; Huang, D.-S.; Donnelly, M. I.; Frey, P. A. *Biochemistry* **1990**, *29*, 1696−1700.