Density Functional Study on Possible Peroxo Form of Non-heme Diiron Enzyme Model

Kazunari Yoshizawa,*† Yasunori Yokomichi,#† Yoshihito Shiota,† Takehiro Ohta,† and Tokio Yamabe*†,††
†*Department of Molecular Engineering, Kyoto University, Sakyo-ku, Kyoto 606-01*
††*Institute for Fundamental Chemistry, 34-4 Takano-Nishihiraki-cho, Sakyo-ku, Kyoto 606*

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Dioxygen binding to the active dinuclear iron site of methane monooxygenase (MMO) is theoretically analyzed with a density functional method. The μ-η^1:η^1-O_2 mode was calculated to be the most favorable binding mode of dioxygen to a supposed active site of MMO that contains two five-coordinate irons(II).

Methane monooxygenase (MMO) hydroxylates methane, the most inert hydrocarbon.[1] The dinuclear non-heme iron site of MMO interacts with dioxygen (O_2) in the early stages of its important catalytic cycle.[2] We have relevant and important structural information through the X-ray structure of MMO from *Methylococcus capsulatus* (Bath).[3] The interesting core structure reported for MMO and other non-heme dinuclear iron enzymes such as various forms of hemerythrin[2] consists of two nearly-octahedrally coordinated iron atoms joined by a μ-oxo, μ-hydroxo, or μ-aqua bridge and one or two μ-carboxylato bridges. In previous papers,[4] we have discussed possible intermediates of MMO and their reactivity with methane using the extended Hückel method. In this Letter, we discuss dioxygen-binding to the dinuclear iron active site of MMO theoretically with a density functional method (ADF)[5] available on Cerius2 program package.[6]

Raman study of intermediate P of MMO[7] exhibited an isotope-sensitive line at 905 cm^{-1} which falls within the O–O stretching frequency range observed in several metal peroxo model complexes (ν(O–O) = 815-918 cm^{-1}). Optical and Raman studies[7,8] suggest that dioxygen is coordinated to MMO symmetrically in fashion μ-η^1:η^1-O_2 or μ-η^2:η^2-O_2. Peroxo model complexes with a μ-η^1:η^1-O_2 mode were recently characterized by the research groups of Suzuki,[9] Que,[10] and Lippard.[11] From the observed Raman line at 905 cm^{-1}, one can imagine that intermediate P is likely to have a μ-η^1:η^1-O_2 mode (*cis*- or *trans*-μ-1,2-O_2 mode).

We assume that the active site of MMO is occupied in the crystal structures known by an exogenic bidentate acetate or aqua bridge, depending on the conditions used to prepare crystals.[3] That extra ligand may not be coordinated to the real active site in the physiologically active state of the enzyme. In fact, in a chemically reduced active site of MMO (H_{red}), a carboxylate shift and loss of the bridging hydroxide and the water or acetate ligand afford essentially two five-coordinate irons(II).[3b] Thus, there may be two vacant pockets in the physiologically active site of MMO.[4]

Our theoretical active-site model contains hydroxo and carboxylato bridges connecting two five-coordinate irons(II). We used OH_2 and NH_3 ligands to model the remainder of the coordination sphere. An uncontracted triple zeta STO basis set with a frozen core (2p) approximation was adopted for Fe. Double zeta basis sets were used for H, C, N, and O atoms, except for O atoms of dioxygen for which a triple zeta basis set plus polarization functions were used. MOLDEN[12] was used for molecular orbital visualization.

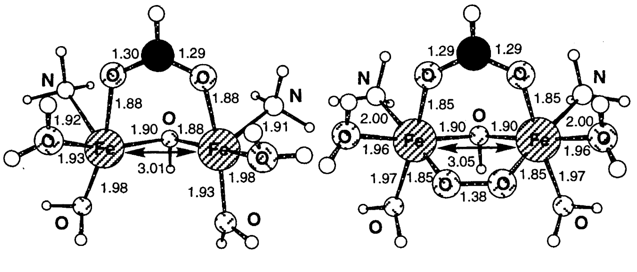

Figure 1. Optimized geometries of the supposed active site and the peroxo form of non-heme diiron enzyme model.

Figure 1 shows the optimized geometries of the supposed active site that has two five-coordinate irons and the peroxo form of a non-heme diiron enzyme model. The Fe····Fe nonbonded distances in the active site with five-coordinate irons and in the peroxo form are 3.01 and 3.05 Å, respectively. The diheral angle of Fe–O–O–Fe is 0°: these atoms are on a plane. Our calculations suggest that a μ-η^1:η^1-O_2 mode is the most favorable binding mode of dioxygen to the active site where one of bridging ligands is removed. This result is fully consistent with X-ray structural analyses of model peroxo complexes[9,10] and our earlier qualitative calculations.[4] The optimized O–O distance of 1.38 Å lies between those of free dioxygen (1.21 Å) and hydrogen peroxide (1.49 Å). Each iron atom is in a nearly-octahedral environment in the peroxo form. This situation is quite different from the well-known planar μ-η^2:η^2-O_2 mode seen for dioxygen binding to hemocyanin,[13-15] an oxygen-activating enzyme which contains dinuclear copper (Cu^I) site at its active site.

The net charge of one iron atom in the optimized peroxo form with an O–O distance of 1.38 Å is -0.28. When we dissociate the O–O bond, the magnitude of the net charge (minus) increases with an increase in bond distance. The net charge is -0.33 and -0.48 for the O–O distance of 1.50 and 2.50 Å, respectively. A significant electron transfer occurs from the d-block of the irons to the coordinating dioxygen in the process of dioxygen bond cleavage.

Figure 2 shows in-phase and out-of-phase combinations of nonbonding d-block orbitals (LUMO and LUMO+1, respectively) of the supposed active site of MMO.[4a] These orbitals point toward the missing water coordination, and the in-phase combination (lower one) interacts with dioxygen π and the out-of-phase one (upper one) interacts nicely with dioxygen π^*, as suggested. While π^* begins in neutral O_2 with two electrons, in the early stages of the reaction two

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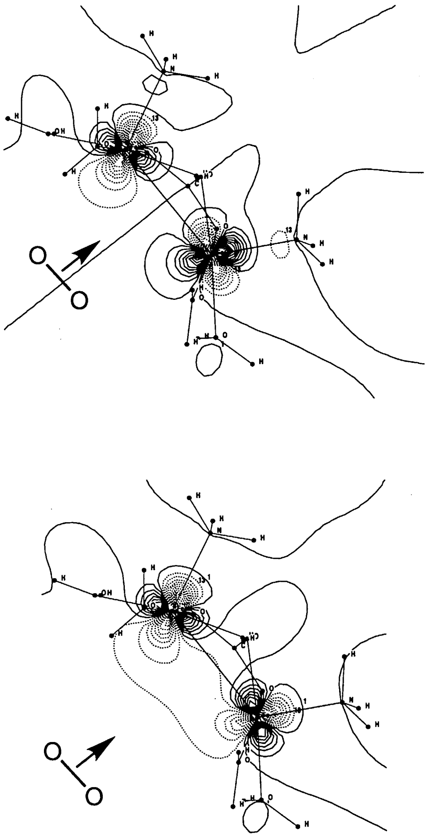

Figure 2. In-phase (lower) and out-of-phase (upper) combinations of nonbonding d-block orbitals (LUMO and LUMO+1, respectively) of the supposed active site of MMO.

electrons are transferred from the high-lying (Fe,Fe) t_{2g} block to O_2, reducing it to O_2^{2-}.

A recent EXAFS and Mössbauer study[16] suggested that intermediate Q which can directly activate methane would have a high-valent $Fe_2(\mu\text{-}O)_2$ diamond core structure. We think from Raman studies[7,8,11] that peroxo intermediate P should have a *cis-* or *trans*-μ-1,2-O_2 mode, so that it is important for a future study to examine how the high-valent $Fe_2(\mu\text{-}O)_2$ diamond core can be created from such a peroxo species.

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Permanent address: Kansai Research Institute, Kyoto Research Park 17, Shimogyo-ku, Kyoto 600.

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