

## Communications

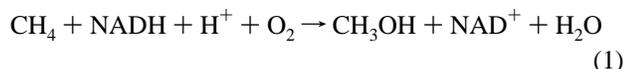
### Dioxygen Binding to Dinuclear Iron Centers on Methane Monooxygenase Models

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The dinuclear non-heme iron centers of hemerythrin (Hr), ribonucleotide reductase (RR), and methane monooxygenase (MMO) interact with dioxygen (O<sub>2</sub>) as a part of their important biochemical function.<sup>1</sup> MMO hydroxylates methane, the most inert hydrocarbon, eq 1.<sup>2</sup> How exactly this is accomplished is

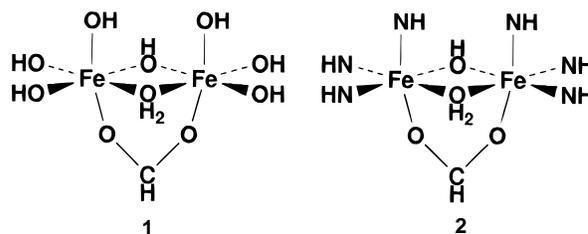


of great interest; we have relevant and important structural information through the X-ray structure of MMO from *Methylococcus capsulatus* (Bath).<sup>3</sup> In this communication we discuss dioxygen binding to the dinuclear iron centers of MMO, making use of an approximate molecular orbital theory.<sup>4</sup>

The interesting core structure reported for MMO and other non-heme iron enzymes mentioned above consists of two nearly-octahedrally coordinated iron atoms joined by a  $\mu$ -oxo,  $\mu$ -hydroxo, or  $\mu$ -aqua bridge and one or two  $\mu$ -carboxylato bridges. Two examples of a  $\mu$ -aqua bridge are known for model complexes.<sup>5</sup> The remainder of the metal coordination sphere is composed of either nitrogen coming from a histidine residue

or oxygen from hydroxyl, carboxyl, and water. EXAFS studies have shown that the average Fe—O/N bond length in MMO is in the range 2.04–2.15 Å,<sup>6</sup> which is not so different from the standard covalent bond lengths, 1.98 and 2.00 Å for Fe—O and Fe—N, respectively.

Our theoretical models of MMO are indicated in **1** and **2**; they contain hydroxo, carboxylato, and aqua bridges connecting two irons. We used OH<sup>-</sup> and NH<sub>2</sub><sup>-</sup> ligands to model the



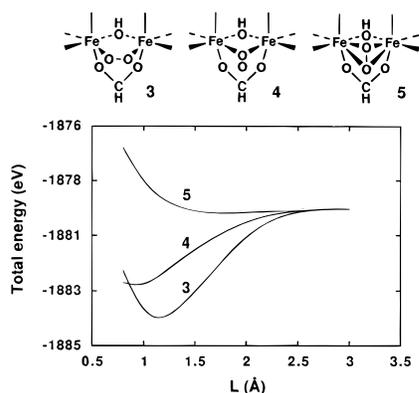
remainder of the coordination sphere. The Fe...Fe distance was taken as 3.1 Å from the most recent X-ray structural analysis,<sup>3b</sup> and the Fe—O/N bond lengths were assumed to be 2.0 Å. The  $\mu$ -hydroxo bridge angle is 101.6° in these structures. Under certain crystallization conditions, it seems that an acetate ligand may also bridge the irons, which are then separated by 3.4 Å.<sup>3a</sup> Each iron atom is in a nearly-octahedral environment; all the bond angles at the iron centers are approximately 90°.

The computed and expected d-orbital splitting of these molecules is a six-below-four pattern, characteristic of octahedrally-coordinated and weakly interacting dimetals.<sup>7</sup> In the reduced form (Fe<sup>II</sup>Fe<sup>II</sup>), which interacts with dioxygen,<sup>1</sup> the low-lying d-orbital block is fully occupied and the high-lying one vacant. We assume that the active site of MMO is occupied in the crystal structures known by an exogenous bidentate acetate or aqua bridge, depending on the conditions used to prepare crystals.<sup>3</sup> That extra ligand may not be coordinated to the active

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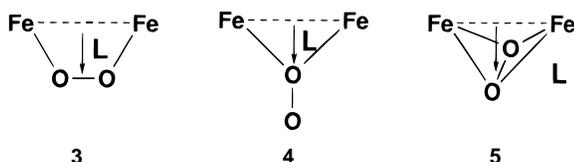


**Figure 1.** Potential energy curves for three types of coordination modes (**3**,  $\mu\text{-}\eta^1\text{:}\eta^1\text{-O}_2^{2-}$ ; **4**,  $\mu\text{-O}_2^{2-}$ ; **5**,  $\mu\text{-}\eta^2\text{:}\eta^2\text{-O}_2^{2-}$ ) of  $\text{Fe}_2(\mu\text{-OH})(\mu\text{-O}_2\text{CH})(\text{OH})_6 + \text{O}_2$ .

centers in the physiologically active state of the enzyme. Thus there may be two vacant (five-coordinate) sites in active MMO.

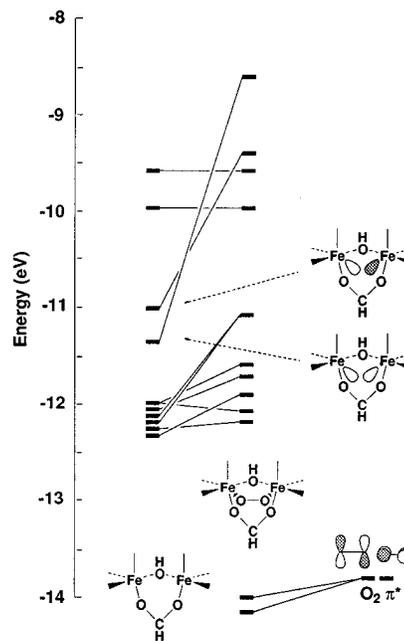
The principal kinetic barrier to direct reaction of dioxygen with an organic substrate arises from the fact that highly reactive singlet dioxygen is 22.5 kcal/mol higher in energy than ground-state triplet dioxygen.<sup>1a</sup> In order to overcome this spin restriction, dioxygen reacts with the reduced form of the enzyme, probably forming a diiron(III) peroxide intermediate in the early stages of the total reaction indicated in eq 1. A recent Raman study of an intermediate of MMO<sup>8</sup> exhibited an isotope-sensitive line at 905  $\text{cm}^{-1}$  which falls within the O–O stretching frequency range observed in several metal peroxo model complexes ( $\nu(\text{O}=\text{O}) = 815\text{--}918\text{ cm}^{-1}$ ). We assumed the bond length of the coordinated dioxygen to be 1.4 Å.<sup>9</sup>

Several binding modes of  $\text{O}_2$  to MMO have been suggested.<sup>10</sup> We considered the following important geometries: **3** ( $\mu\text{-}\eta^1\text{:}\eta^1\text{-O}_2^{2-}$ ), **4** ( $\mu\text{-O}_2^{2-}$ ), and **5** ( $\mu\text{-}\eta^2\text{:}\eta^2\text{-O}_2^{2-}$ ). These structures



are quite different from that of Hr, in which dioxygen is asymmetrically and reversibly bound to the five-coordinate iron to form oxyHr.<sup>1</sup>

Optical and Raman studies<sup>8,11</sup> suggest that dioxygen is coordinated to MMO symmetrically in fashion **3** or **5**, although the spectroscopic characteristics of the actual peroxo complex of MMO<sup>8</sup> differ from those of a model complex<sup>11</sup> in several respects. Some possible structures of intermediates have been discussed on the basis of Mössbauer and other spectroscopic measurements.<sup>12</sup> It is not easy to determine, from these spectroscopic data alone, which mode is actually found. Figure 1 shows the computed potential energy curves for the three binding modes, (a)  $\mu\text{-}\eta^1\text{:}\eta^1\text{-O}_2^{2-}$ , (b)  $\mu\text{-O}_2^{2-}$ , and (c)  $\mu\text{-}\eta^2\text{:}\eta^2\text{-O}_2^{2-}$ , of the hydroxide ligand model, **1**.  $L$  is the distance



**Figure 2.** Interaction diagram of the most favorable mode (**3**) of dioxygen binding to  $\text{Fe}_2(\mu\text{-OH})(\mu\text{-O}_2\text{CH})(\text{OH})_6$ .

between the center of the line joining the two irons and the coordinated dioxygen, as defined in **3**, **4**, and **5**.

Our calculations suggest that **3** is the most favorable binding mode of dioxygen to the active site where a  $\mu$ -aqua bridge is removed. The results obtained for the imide ligand model **2** are virtually identical; the minima are just a little shallower. The binding energy computed, not absolutely reliable in the extended Hückel method, is approximately 3–4 eV relative to  $\text{Fe}_2(\mu\text{-OH})(\mu\text{-O}_2\text{CH})(\text{NH})_6$  or  $\text{Fe}_2(\mu\text{-OH})(\mu\text{-O}_2\text{CH})(\text{OH})_6 + \text{O}_2$ . In contrast to **3**, there is no stabilization energy in **5**. This situation is quite different from the well-known planar  $\mu\text{-}\eta^2\text{:}\eta^2\text{-O}_2^{2-}$  mode seen for dioxygen binding to hemocyanin,<sup>9,13–16</sup> an oxygen-activating enzyme which contains dinuclear copper ( $\text{Cu}^I$ ) centers at its active site.

Figure 2 shows the orbital interaction diagram for the most favorable dioxygen-binding mode, **3**. Note the block of six  $t_{2g}$ -like orbitals of the two metal centers at  $\sim -12$  eV, followed in rising energy by the two Lewis-acid sites, orbitals pointing toward the missing water coordination. These orbitals interact well with the dioxygen  $\pi^*$  (and  $\pi$ ). While  $\pi^*$  begins in neutral  $\text{O}_2$  with two electrons, we think that in the early stages of the reaction two electrons are transferred from the high-lying Fe, Fe  $t_{2g}$  block to  $\text{O}_2$ , reducing it to  $\text{O}_2^{2-}$ .

Our calculations suggested that the  $\mu\text{-}\eta^1\text{:}\eta^1\text{-O}_2^{2-}$  mode is the most favorable mode for binding of  $\text{O}_2$  to MMO. A detailed analysis, to be published elsewhere, will trace the consequences of this coordination in methane activation.

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