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Structural Model Studies for the High-Valent Intermediate Q of Methane Monooxygenase from Broken-Symmetry Density Functional Calculations

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Abstract

Mössbauer isomer shift parameters have been obtained for both density functional theory (DFT) OPBE and OLYP functionals by linear regressions between the measured isomer shifts and calculated electron densities at Fe nuclei for a number of $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes grouped separately. The calculated isomer shifts and quadrupole splittings on the sample Fe complexes from OPBE and OLYP functionals are similar to those of PW91 calculations (J. Comput. Chem. 27 (2006) 1292), however the fit parameters from the linear regressions differ between PW91 and OPBE, OLYP. Four models for the active site structure of the hydroxylase component of soluble methane monooxygenase (MMOH) have been studied, using three DFT functionals OPBE, OLYP, and PW91, incorporated with broken-symmetry methodology and the conductor-like screening (COSMO) solvation model. The calculated properties, including optimized geometries, electronic energies, pK_a 's, Fe net spin populations, and Mössbauer isomer shifts and quadrupole splittings, have been reported and compared with available experimental values. The high-spin antiferromagnetically (AF) coupled Fe^{4+} sites are correctly predicted by OPBE and OLYP methods for all active site models. PW91 potential overestimates the Fe-ligand covalencies for some of the models because of spin crossover. Our calculations and data analysis support the structure (our current model II shown in Figure 8) proposed by Friesner and Lippard's group (J. Am. Chem. Soc. 123 (2001) 3836–3837), which contains an $\text{Fe}^{4+}(\mu\text{-O})_2\text{Fe}^{4+}$ center, one axial water which also H-bonds to both side chains of Glu243 and Glu114, and one bidentate carboxylate group from the side chain of Glu144, which is likely to represent the active site of MMOH-Q. A new model structure (model IV shown in Figure 9), which has a terminal hydroxo and a protonated His147 which is dissociated from a nearby Fe, is more asymmetric in its $\text{Fe}(\mu\text{-O})_2\text{Fe}$ diamond core, and is another very good candidate for intermediate Q.

Keywords

Density Functional Theory; Methane Monooxygenase; Intermediate Q; Fe-oxo Active Site; Mössbauer Isomer Shift Fitting; Quadrupole Splitting

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1. Introduction

Soluble methane monooxygenase (MMO) catalyzes the hydroxylation of methane, which requires cleavage of stable and non-polar C-H bond in methane (converting methane to methanol) [1-3]. The MMO enzyme consists of three components: a hydroxylase protein (MMOH); a regulatory protein for molecular oxygen binding, called component B (MMOB); and a reductase (MMOR), which is a 2Fe2S protein that provides two electrons to the hydroxylase. MMOH contains a carboxylate-bridged non-heme diiron center at its active site and catalysis the transformation of methane and oxygen to methanol and water. The reaction cycle of MMOH with methane or other alkanes can be summarized by the following set of reactions:

where R = methane or the higher alkanes. This simple sequence of reaction steps belies the structural and energetic complexities associated with the catalytic process. Coupled electron and proton addition to the $\text{Fe}^{3+}\text{Fe}^{3+}$ resting enzyme generates the reduced $\text{Fe}^{2+}\text{Fe}^{2+}$ state. Two spectroscopically observed intermediates, P and Q, are then formed upon reaction with dioxygen. Despite the collective experimental effort, structures of these intermediate species are not yet available. P was found to be a peroxo- $\text{Fe}^{3+}\text{Fe}^{3+}$ complex by resonance Raman spectroscopy [1,4]. Q is the species that reacts with methane to form methanol, and is proposed to have two near-equivalent Fe^{4+} sites that are antiferromagnetically (AF) coupled with $J < -30 \text{ cm}^{-1}$ by Mössbauer spectroscopy (spin Hamiltonian $H = -2JS_1 \cdot S_2$) [5,6]. For MMO in *Methylosinus trichosporium* (Mt) OB3b system, the Mössbauer spectrum of Q is symmetrical with isomer shifts (δ) and quadrupole splittings (ΔE_Q) of ($\delta = 0.17 \text{ mm s}^{-1}$, $\Delta E_Q = 0.53 \text{ mm s}^{-1}$) for both iron sites [5]. The spectrum of Q in *Methylococcus capsulatus* (Mc) (Bath) system is a quadrupole doublet which can be fit with two unresolved equal intensity quadrupole doublets ($\delta = 0.21 \text{ mm s}^{-1}$ and $\Delta E_Q = 0.68 \text{ mm s}^{-1}$ for doublet 1 and $\delta = 0.14 \text{ mm s}^{-1}$ and $\Delta E_Q = 0.55 \text{ mm s}^{-1}$ for doublet 2) [6]. The X-ray absorption experiments on Mt indicate each Fe^{4+} site of Q samples has a pre-edge area of 28 units. Such a large value implies that the Fe^{4+} centers in Q have a highly distorted geometry and are likely to have a coordination number no greater than 5 [7]. The EXAFS (extended X-ray absorption fine structure) measurements and analysis on Q also suggest a diamond core (Fe_2O_2) exists comprising one short (1.77 Å) and one long (2.05 Å) bond to each iron and a short Fe-Fe distance ranging from 2.46 to 2.52 Å [7,8].

Several active site structural models of Q have been studied theoretically by different groups [9-19], which were reviewed by Baik et al. [17]. Many earlier models did not consider the antiferromagnetic spin coupling between the Fe^{4+} sites. Figure 1 shows two currently promising active site structures of Q which were proposed by Siegbahn (Figure 1a) [11,12] and by Friesner and Lippard's group (Figure 1b) [16,17]. The two-bidentate bridging carboxylate model (Figure 1a) produced shorter Fe-Fe distance, which is more consistent with the EXAFS data analysis. The one-bidentate bridging carboxylate model (Figure 1b), however, is geometrically closer to both the reduced and oxidized MMOH X-ray crystal structures [20, 21]. From both models, the energetics associated with methane (and other alkanes) binding and activation have been explored [12,16,22-25].

In attempting to construct a quantum chemical model that accurately represents intermediate Q, our working hypothesis is that the active site cluster should be consistent with most of the experimental (Mössbauer and EXAFS) observations noted above. Our group was the first to compute the Mössbauer isomer shift and quadrupole splitting properties for cluster models of Q. We have reevaluated the two proposed models in Figure 1 with increased size to include the second shell H-bonding residue sidechains and some explicitly H-bonding water molecules. The structures are shown in Figure S3 of reference [18] and Figure 31 of reference [19].

The isomer shifts were originally calculated according to the fit equation for density functional PW91 [26] potential with all-electron triple- ζ plus polarization (TZP) Slater type basis sets [18,27,28],

$$\delta = \alpha (\rho(0) - 11884.0) + C \quad (4)$$

where $\rho(0)$ is the electron density at Fe nucleus and ($\alpha = -0.664$, $C = 0.478 \text{ mm s}^{-1}$) were obtained by our first linear fitting between the calculated $\rho(0)$ and experimental values δ for totally 21 dinuclear and mononuclear Fe^{2+} , Fe^{3+} , and Fe^{4+} complexes [18,27]. The isomer shifts for the high-spin AF-coupled Fe^{4+} sites of our larger MMOH-Q models were then calculated. They are 0.35 and 0.07 mm s^{-1} for the two-bidentate bridging carboxylate model [18], and are 0.38 and -0.03 mm s^{-1} for the one-bidentate bridging carboxylate model. The isomer shift difference ($\Delta\delta$) of the one-bidentate bridging carboxylate model is obviously much larger than the two-bidentate model. We therefore drew the conclusion that the two-bidentate model is better than the one-bidentate model in reproducing both the experimental Mössbauer and EXAFS data [18,19].

However, later we noticed a problem with our previous global fitting that, the isomer shift predictions underestimate the Fe^{2+} and Fe^{4+} sites, but in general overestimate the Fe^{3+} state (see Figure 1 and Table 1 of reference [27]). Our hypothesis is that the electron-rich ferrous Fe is much more sensitive than Fe^{3+} and Fe^{4+} to its ligands and solvent (counterion) environment. There is possible charge transfer from the Fe^{2+} center (involving the first shell ligands) to the environment, which cannot be calculated without including the explicit solvent molecules. The shift of the electron density at Fe^{2+} nuclei is therefore underestimated [29]. In order to reasonably predict the ^{57}Fe isomer shifts in different oxidation states, one needs to fit the parameters separately for Fe^{2+} and $\text{Fe}^{3+,4+}$ complexes [29,30]. Another disadvantage of this fitting is that, only gas-phase single-point energy calculations were performed directly on the geometries of the sample complexes taken from the Cambridge Structural Database (CSD) [31,32] (only the hydrogen atom positions were optimized). Though the geometries of these small molecules are of high resolution, they are not necessarily at the minimum on the potential energy surface of the specified PW91 potential. The gas-phase calculations also neglect solvation effects. We then increased the size of the training set and reperformed the fittings for the $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes separately [29]. The geometries and electronic structures of all complexes in the training set were optimized within the conductor like screening (COSMO) [33-35] solvation model (with dielectric constant $\epsilon = 32.7$, methanol) in the Amsterdam Density Functional (ADF) [30,36-38] package. The COSMO model is a dielectric solvent continuum model in which the solute molecule is embedded in a molecular shaped cavity surrounded by a dielectric medium with a given dielectric constant.

The new PW91 fitting for 17 $\text{Fe}^{2+,2.5+}$ complexes (totally 31 Fe sites) yields $\alpha = -0.405 \pm 0.042$ and $C = 0.735 \pm 0.047 \text{ mm s}^{-1}$. The correlation coefficient is $r = -0.876$ with a standard deviation $\text{SD} = 0.075 \text{ mm s}^{-1}$ [29]. In contrast, the linear fitting for 19 $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes (totally 30 Fe sites) yields $\alpha = -0.393 \pm 0.030$ and $C = 0.435 \pm 0.014 \text{ mm s}^{-1}$ (with $r = -0.929$ and $\text{SD} = 0.077 \text{ mm s}^{-1}$) [29,30]. Using these new parameters, predictions of isomer shifts are much improved for the active sites of both the oxidized diferric and reduced diferrous state MMOH and class-I ribonucleotide reductase (RNR) [29]. Now with $\alpha = -0.393$ and $C =$

0.435 mm s⁻¹, we recalculated the isomer shifts for our Q models mentioned above, and obtained $\delta = 0.36$ and 0.19 mm s⁻¹ for our two-bidentate bridging carboxylate model [29], and $\delta = 0.38$ and 0.14 mm s⁻¹ for our one-bidentate bridging carboxylate model. The situation becomes uncertain. The predicted isomer shifts of the two models now are very close to each other. It is difficult to judge which model is more favored than the other. This motivates us to perform further studies on potential structures of intermediate Q, by including more H-bonding residue side chains into the model cluster and investigating more structural models. In our previous geometry optimizations, all relative atomic positions of the outer shell residues to the Fe centers were fixed according to the X-ray crystal structure. By contrast, we now perform full geometry optimization including COSMO solvation with only the linking H atom [39] positions of the outer shell residues fixed.

We first used PW91 potential for the calculations, and found out that the net spin populations on the two iron sites for the large one-bidentate bridging carboxylate model are below 2. The Fe-ligand covalencies are over estimated. Also the predicted quadrupole splitting values are much larger than the observed ones. The PW91 potential has been demonstrated to be very accurate in predicting various properties in our previous studies. However, it may fail to produce the high-spin AF-coupled diiron state in the region near spin crossover.

Validation of exchange-correlation (xc) DFT functionals for predicting correct spin ground state of iron complexes has not been widely explored. Swart et al. have reported a systematic study on the performance of several exchange-correlation functionals for seven iron complexes which are experimentally found to have either a low, intermediate, or high spin ground state [40]. They found that the Handy's optimized exchange (OPTX) [41] functional performs well, and the OPBE functional, which is the combination of OPTX and PBE correlation (PBEc) [42,43], performs the best [40] among GGA-type potentials (GGA potentials are more computationally efficient than hybrid Hartree-Fock GGA potentials when density fitting procedures are used). These authors have also tested different functionals in calculating the atomization energies for the G2-set of up to 148 molecules, six reaction barriers of S_N2 reactions, geometry optimizations of 19 small molecules and 4 metallocenes, and zero-point vibrational energies for 13 small molecules [44]. Their examination shows that the OPTX containing functionals perform better than the regular general gradient approximation functionals (GGAs) like PBE [42,43], BLYP [45,46], and BP [45,47,48]. OPBE performs exceptionally well in all cases, and is as good as OLYP, which for organic systems has been shown to function as well as the hybrid functional B3LYP [44,49]. OLYP is a combination of OPTX and LYP correlation [46]. We therefore also applied the two GGA functionals OPBE and OLYP in the current study, in order to locate the potential structure for MMOH-Q by comparing the properties calculated by the different functionals at the active site models with the available experimental data.

For calculating the isomer shifts using OPBE and OLYP functionals, we performed the linear fitting on the same training sets of Fe^{2+,2.5+} and Fe^{2.5+,3+,3.5+,4+} complexes as we did for PW91 potential [29]. The fitting results will be given in section 2. The model clusters and the calculational details for the MMOH intermediate Q will be given in section 3. Results and discussion then follow in section 4.

2. Mössbauer Isomer Shift and Quadrupole Splitting Calculations

2.1. New Liner Fitting for Isomer Shifts Using OPBE and OLYP Functionals

The isomer shift is proportional to the electron density ($\rho(0)$) difference at the Fe nuclei between the studied and a reference system (normally α -Fe at 300 K), and can be described as [27,29, 50,51]:

$$\delta = \alpha (\rho(0) - b) \quad (5)$$

or

$$\delta = \alpha (\rho(0) - A) + C \quad (6)$$

where A is a constant chosen close to the electron density at the Fe nucleus in the reference state. $\rho(0)$ is calculated for a set of Fe complexes (whose isomer shifts (δ_{exp}) are known from experiment) from the density functional theory, and α and C can be obtained by a linear regression between the calculated $\rho(0)$ and δ_{exp} . The isomer shifts of other Fe compounds then can be predicted. The detailed fitting procedure and the sample $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes and relevant literature citations are given in reference [29]. We now have reoptimized the geometries of these iron complexes using the OPBE and OLYP functionals with triple- ζ plus polarization (TZP) Slater type basis sets (the inner core shells of C(1s), N(1s), O(1s) and Fe(1s,2s,2p) were frozen) within COSMO solvation model in ADF2005.01 with a reasonable polar environment $\epsilon = 32.7$ (the dielectric constant for methanol) [30]. Then the COSMO single-point energy calculations with all-electron TZP Slater type basis sets are performed on the optimized geometries to calculate the $\rho(0)$.

The experimental Fe isomer shifts (δ_{T}) were taken at different temperatures (T). We therefore shift them to a common temperature of 4.2 K, by taking account of the second-order Doppler effect. The offset given by ($\delta_{4.2\text{K}} - \delta_{300\text{K}}$) for this correction is approximately 0.12 mm s^{-1} , and this is expected to be linear with temperature [27,29]. The OPBE and OLYP calculated $\rho(0)$, the shifted $\delta_{4.2\text{K}}$ for our $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ training sets are given in Tables 1 and 2, respectively. The details of the compounds, the experimental temperature, and the measured δ_{T} have been given in reference [29]. Based on the correlation

$$\delta_{4.2\text{K}} = \alpha (\rho(0) - 11877.0) + C \quad (7)$$

the new linear regressions for the $\text{Fe}^{2+,2.5+}$ complexes (see Figures 2 and 3, totally N = 31 Fe sites) yield $\alpha = -0.318 \pm 0.049$ and $C = 0.633 \pm 0.084 \text{ mm s}^{-1}$ for OPBE functional, and $\alpha = -0.328 \pm 0.052$ and $C = 0.622 \pm 0.089 \text{ mm s}^{-1}$ for OLYP functional. The correlation coefficient is $r = -0.772$ with a standard deviation $\text{SD} = 0.098 \text{ mm s}^{-1}$ for OPBE, and $r = -0.760$ with $\text{SD} = 0.101 \text{ mm s}^{-1}$ for OLYP. Similarly, the linear regressions for our $\text{Fe}^{2.5+,3+,3.5+,4+}$ training set (totally N = 30 Fe sites) yield $\alpha = -0.312 \pm 0.022$ and $C = 0.373 \pm 0.014 \text{ mm s}^{-1}$ with $r = -0.939$ and $\text{SD} = 0.072 \text{ mm s}^{-1}$ for OPBE, and $\alpha = -0.307 \pm 0.023$ and $C = 0.385 \pm 0.015 \text{ mm s}^{-1}$ with $r = -0.931$ and $\text{SD} = 0.076 \text{ mm s}^{-1}$ for OLYP potential.

Using the new fitted parameters α and C, the calculated isomer shifts ($\delta_{\text{cal}} = \alpha(\rho(0) - 11877.0) + C$) for these $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes are also obtained for both the OPBE and OLYP calculations and are given in Tables 1 and 2. As seen in Figures 2 and 3 and Tables 1 and 2, calculations based on OPBE and OLYP yield similar results. As observed in the corresponding linear fittings for PW91 potential [29], the slopes of the $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ linear regressions are essentially the same for both OPBE and OLYP. However the intercepts of the two fitted lines are different, and the vertical shift between the two linear regressions is about 0.26 mm s^{-1} for OPBE and 0.24 mm s^{-1} for OLYP. This difference may come from the possible charge transfer from the electron-rich Fe^{2+} center (involving the first shell ligands) to the solvent (and counterion) environment which cannot be calculated even using the COSMO solvation model. As a result, the electron densities at the Fe^{2+} nuclei are overestimated (or the isomer shifts at Fe^{2+} nuclei are underestimated). A larger y intercept (isomer shift axis) for the $\text{Fe}^{2+,2.5+}$ complexes is therefore needed to compensate this deficiency of the calculation.

The two $\text{Fe}^{2.5+}$ sites in $\text{Fe}_2(\text{salmp})_2^-$ are included in both training sets. The calculated isomer shifts (the same for both OPBE and OLYP calculations, see Tables 1 and 2) of these $\text{Fe}^{2.5+}$ sites obtained from the $\text{Fe}^{2+,2.5+}$ fitting are larger than the experimental values by 0.14 mm s^{-1} , and the corresponding results obtained from the $\text{Fe}^{2.5+,3+,3.5+,4+}$ linear fitting are smaller

than the experimental data by 0.12 mm s^{-1} . The averaged calculated values (0.84 mm s^{-1} for both sites) are almost exactly the experimental data (0.83 mm s^{-1} at 4.2 K), which is very similar to the case obtained from PW91 potential [29]. Using the averaged calculated isomer shifts for the two $\text{Fe}^{2.5+}$ sites, we performed the linear regression between the calculated and experimental isomer shifts for all the Fe complexes given in Tables 1 and 2 for both the OPBE and OLYP potentials; the correlation plots of $\delta_{4.2\text{K}} = A\delta_{\text{cal}} + B$ are shown in Figure 4.

These linear fittings yield the slopes $A = 1.007 \pm 0.026$ for OPBE and $A = 1.007 \pm 0.027$ for OLYP, which are almost the ideal value 1.0; and the intercepts $B = -0.006 \pm 0.023$ (OPBE) and $B = -0.006 \pm 0.024$ (OLYP) mm s^{-1} which are also around the ideal value zero. The correlation coefficient of the fitting for OPBE is $r = 0.982$ with a standard deviation of $\text{SD} = 0.079 \text{ mm s}^{-1}$, and $r = 0.981$ with $\text{SD} = 0.082 \text{ mm s}^{-1}$ for OLYP. The standard deviations of these fittings for OPBE and OLYP potentials are slightly larger than that (0.066 mm s^{-1}) for PW91 potential [29]. Overall, the accuracies of the fittings for OPBE and OLYP potentials are very similar to those for PW91 potential and are in good agreement with the experiments.

2.2. OPBE and OLYP Calculated Quadrupole Splittings for the Sample Fe complexes

The quadrupole splitting (ΔE_Q) of an Fe atom arises from the non-spherical nuclear charge distribution in the $I = 3/2$ excited state, and is proportional to the electric field gradient (EFG) at the Fe nucleus which can be calculated directly from ADF. The EFG tensors (V) were obtained by a COSMO single-point energy calculation using all-electron TZP basis sets at the COSMO optimized geometries. V is diagonalized and its eigenvalues are reordered so that $|V_{zz}| \geq |V_{xx}| \geq |V_{yy}|$. The asymmetry parameter η is then defined as

$$\eta = |(V_{xx} - V_{yy})/V_{zz}| \quad (8)$$

Finally the quadrupole splitting for ^{57}Fe of the nuclear excited state ($I = 3/2$) can be calculated as

$$\Delta E_Q = \frac{1}{2}eQV_{zz}\left(1 + \eta^2/3\right)^{\frac{1}{2}} \quad (9)$$

where e is the electrical charge of a positive electron, Q is the nuclear quadrupole moment (0.15 barns) of Fe [52].

The OPBE and OLYP calculated quadrupole splittings ($\Delta E_{Q(\text{cal})}$) and η for our 35 sample Fe complexes (59 Fe sites in total) are also given in Tables 1 and 2, and are compared with the experimental data ($\Delta E_{Q(\text{exp})}$). The linear correlations (see Figure 5) between the calculated and the observed ΔE_Q absolute values based on the equation

$$|\Delta E_{Q(\text{exp})}| = A|\Delta E_{Q(\text{cal})}| + B \quad (10)$$

yield $A = 0.986 \pm 0.030$ and $B = 0.110 \pm 0.067 \text{ mm s}^{-1}$ for OPBE functional, and $A = 0.972 \pm 0.033$ and $B = 0.117 \pm 0.074 \text{ mm s}^{-1}$ for OLYP. The correlation coefficients are $r = 0.975$ (OPBE) and $r = 0.969$ (OLYP) with the standard deviations $\text{SD} = 0.249 \text{ mm s}^{-1}$ (OPBE) and $\text{SD} = 0.277 \text{ mm s}^{-1}$ (OLYP). The SD value obtained from OPBE is almost the same as that (0.247 mm s^{-1}) obtained from PW91 calculations [29]. The SD predicted by OLYP is only by 0.03 mm s^{-1} worse than the other two methods for these sets of complexes. Experimentally the signs of the quadrupole splittings were not reported for many of these complexes. Theoretically we observed that the signs of the quadrupole splittings may vary with methods of calculation and the basis sets used in the calculations. Of course if the asymmetry parameter η is close to 1, the sign of the quadrupole splitting can be easily changed by the environment and the different computational methods. We therefore mainly focused on the absolute values of the quadrupole splittings when comparing our calculations with experiment.

The compound $\text{Fe(II)Fe(III)BPMP(OPr)}_2^{2+}$ contains a diiron center of Fe^{2+} and Fe^{3+} where high-spin sites are AF-coupled yielding a $S_{\text{total}} = \frac{1}{2}$ ground state [53]. The observed quadrupole splittings for the two iron sites are very different (2.69 mm s^{-1} for Fe^{2+} and 0.50 mm s^{-1} for Fe^{3+}). All three exchange-correlation potentials predict a big difference between the two sites. However, the PW91 method yields a much larger quadrupole splitting value for the Fe^{3+} site (1.11 mm s^{-1}) [29] than the corresponding OPBE (0.63 mm s^{-1}) and OLYP (0.68 mm s^{-1}) predictions; while the experimental value is 0.50 mm s^{-1} . For the Fe^{2+} site, the calculated quadrupole splittings are very similar for OPBE, OLYP, and PW91 (2.96 , 2.99 , and 2.92 mm s^{-1}), and are in good agreement with experiment 2.69 mm s^{-1} .

Overall, for OPBE and OLYP functionals, our predictions of the isomer shifts and quadrupole splittings for these structurally well-defined Fe complexes are in very good agreement with the experiments, and are very similar to the corresponding results obtained from PW91 potential.

3. Models and Calculations for the Active Site of MMOH-Q

Four structural models for the active site of MMOH-Q are studied here. The initial geometries of these models are taken or modified from the oxidized diferric crystal structure of MMOH from Mt OB3b protein [21]. The central diiron structures of the first two models are the same as those in Figure 1. In addition to the first-shell residues, we also included several second and third shell H-bonding residue side chains or main chain fragments. Normally a $\text{C}_\beta\text{--C}_\alpha$ or $\text{C}_\gamma\text{--C}_\beta$ bond is broken and a linking hydrogen atom is added to fill the open valence of the terminal carbon atom [39]. These residues are: Glu114, His147, Glu144, His246, Glu209, Glu243, Gln140, Asn249, Asp143, Arg245, Asp242, Arg146, Ser238, and Val239 (see Figures 6-10). Some explicit water molecules are also included in the model clusters. Our model I is again the two-bidentate bridging carboxylate model. The whole model I is shown in Figure 6, and its central diiron structure is given in Figure 7. The second and third shell residues of models II-IV are the same as those in model I (Figure 6). Therefore we only show the diiron core structures of models II-IV in Figures 8, 9 and 10, respectively. Model II is again the so called one-bidentate bridging carboxylate model. A cluster of water molecules were observed in the active site diferric Mt protein in the space between residues Glu209 and Glu114. We included three explicit H-bonding water molecules in the diiron center of model I, so that the numbers of water molecules in models I and II are the same.

Model III is a new model which has not been studied before. The terminal water molecule bonding to Fe1 in model II is replaced by a terminal hydroxo in model III. This idea originated from a private communication of Siegbahn with one of us (LN). Model IV is generated by moving a water proton from O3 to His147-N1, or equivalently by adding a proton to His147-N1 of model III.

All models were geometry optimized within the COSMO [33-35] solvation model with dielectric constant $\epsilon = 80.0$ in ADF2005.01 [30,36-38] using broken-symmetry (BS) [30,54] density functional theory (DFT) and TZP Slater type basis sets (the inner core shells of C(1s), N(1s), O(1s) and Fe(1s,2s,2p) were treated by the frozen core approximation). All linking H atoms in the outer shell residue fragments Asn249, Arg245, Asp143, Asp242, Arg146, Ser238, and Val239, are fixed during the geometry optimizations according to the Cartesian coordinates in the crystal structure. We initially used PW91 potential for the calculations, and found out that the high-spin AF-coupled (or BS) states ($S_{\text{Fe1}} = S_{\text{Fe2}} = 2$, and $S_{\text{total}} = 0$) of models II and III cannot be obtained. The net spin populations on the two iron sites are below 2. The PW91 potential over estimates the Fe-ligand covalency for these models (yielding very large calculated quadrupole splitting values, see next section). As mentioned in the introduction section, we therefore also applied OPBE and OLYP methods in our calculations. The high-spin AF-coupled (or BS) states are easily converged for all models with OPBE and OLYP

potentials. The OPBE geometry optimized structures (within COSMO) of our model clusters for Q were shown in Figures 6-10. Mössbauer isomer shifts and quadrupole splittings were then obtained from COSMO single-point energy calculations on the optimized geometries using TZP Slater type basis sets without freezing the core electrons.

The BS state ($S_{\text{Fe1}} = S_{\text{Fe2}} = 2$, and $S_{\text{total}} = S_{\text{min}} = |S_{\text{Fe1}} - S_{\text{Fe2}}| = 0$) (with energy E_{BS}) obtained from DFT calculations is a mixture of pure spin states. To estimate the energy (E_0) of the pure spin ground state, we also performed a high-spin ferromagnetic (F) coupling COSMO single-point energy calculation ($S_{\text{Fe1}} = S_{\text{Fe2}} = 2$, and $S_{\text{total}} = S_{\text{max}} = |S_{\text{Fe1}} + S_{\text{Fe2}}| = 4$) on the BS state optimized geometry, and obtained the energy E_{F} . When the following Heisenberg (with Heisenberg coupling J) Hamiltonian H is applicable,

$$H = -2JS_1 \cdot S_2 \quad (11)$$

the energy difference between F-coupling and BS states can be described by

$$E_{\text{F}} - E_{\text{BS}} = -4JS_1S_2 \quad (12)$$

And the pure-spin ground state energy E_0 for the particular spin state (S_1, S_2) coupled to S_{min} according to the broken-symmetry geometry is estimated as:

$$\begin{aligned} E_0 &= E_{\text{F}} + JS_{\text{max}}(S_{\text{max}} + 1) - JS_{\text{min}}(S_{\text{min}} + 1) \\ &= E_{\text{F}} + 20J \end{aligned} \quad (13)$$

where $S_{\text{max}} = 4$ and $S_{\text{min}} = 0$ for the current system.

The terminal hydroxo in model III could have another orientation to have H-bonding interaction with O10 in Glu114. However, our OPBE calculations show that this structure has higher energy than the one in Figure 9 where the hydroxo H-bonds to O9 of Glu243. We therefore only present the results of Figure 9 as model III in this paper. To examine whether a terminal water or terminal hydroxo is favored on site Fe1, we also calculated the $\text{p}K_{\text{a}}$ for the terminal water in the process {model II $\rightarrow$ model III + H^+ }. The detailed method for $\text{p}K_{\text{a}}$ calculations have been given in reference [30]. In short, the $\text{p}K_{\text{a}}$ of the terminal water in model II can be calculated by

$$\begin{aligned} 1.37\text{p}K_{\text{a}} &= E_0(\text{model III}) - E_0(\text{model II}) + E(\text{H}^+) \\ &\quad + \Delta G_{\text{sol}}(\text{H}^+, 1\text{atm}) - T\Delta S_{\text{gas}}(\text{H}^+) + \Delta\text{ZPE} + 5/2RT \end{aligned} \quad (14)$$

where $E_0(\text{model III})$ and $E_0(\text{model II})$ represent the energies (after spin-projection) of models III and II, respectively. $E(\text{H}^+)$ is the calculated energy of a spin restricted proton obtained from gas-phase DFT calculation. $E(\text{H}^+) = 12.64, 12.62$, and 12.52 eV, for OPBE, OLYP, and PW91 potentials, respectively. For comparison, we also calculated the physically relevant spin polarized hydrogen ionization potentials (IP) [$E(\text{H}^+) - E(\text{H})_{\text{unrestricted}}$], which are 13.72 eV (OPBE), 13.56 eV (OLYP), and 13.64 eV (PW91), and are all in good agreement with the Rydberg constant 13.6058 eV giving the H atom IP (for infinite nuclear mass). $\Delta G_{\text{sol}}(\text{H}^+, 1\text{atm}) = -262.11$ kcal/mol is the solvation free energy of a proton at 1 atm pressure, and the translation entropy contribution to the gas-phase free energy of a proton is taken as $-T\Delta S_{\text{gas}}(\text{H}^+) = -7.76$ kcal/mol at 298 K and 1 atm pressure [30,55-57]. The zero point energy (ZPE) difference term, $\Delta\text{ZPE} = -7.31$ kcal/mol, was taken from our previous vibrational ZPE calculations for the simplified active site models of RNR intermediate state X [30].

The optimized structure of this model III (Figure 9) obtained from OPBE calculation shows a very long distance ($2.486 \text{ \AA}$) between Fe1 site and N1 of His147, indicating that the His147 side chain does not bind to Fe1 anymore when the terminal water molecule (O3) changes to a terminal hydroxo. We then wondered if the site N1 can be protonated when a terminal hydroxo binds to site Fe1. Therefore model IV was created by adding a proton to N1 in model III. OPBE geometry optimization showed that the His147-N1H group moved upward and H-bonded to

the terminal hydroxo (O3H). This structure also stays unaltered in the OLYP and PW91 COSMO geometry optimizations. The calculated geometries, energies, and Mössbauer properties of models I-IV obtained from OPBE, OLYP, and PW91 potentials are given in Tables 3-5, and will be analyzed and compared with available experimental results in the following section.

4. Results and Discussion

Comparing the geometries for each model obtained from the three exchange-correlation potentials, PW91 potential produces the shortest Fe-Fe distances, and usually shorter Fe-ligand distances and much stronger hydrogen bonding interactions than the other two methods. The bond lengths produced by OPBE potential are normally in between those obtained by PW91 and OLYP calculations.

The computed pK_a of the axial water for the process {model II $\rightarrow$ model III + H^+ } is very large as obtained from OPBE (14.78) and OLYP (11.72) calculations, showing that model III, which has an axial hydroxo, is not favored in these two methods. This pK_a is much smaller (6.98) in PW91 calculations. If the E_{BS} rather than E_0 energies are used in Eq. 14, the pK_a will become a little larger (7.52); both E_0 and E_{BS} show that model II and model III may co-exist in PW91 calculations. However, as mentioned earlier, the net spin populations of the Fe sites (Table 5) in these two models obtained from PW91 potential are small (1.92 — 1.96). Their calculated Heisenberg J values are very negative and their quadrupole splittings (1.73 — 2.68 mm s⁻¹) are much larger than the experimental data (0.53 — 0.68 mm s⁻¹) [5,6]. It seems the PW91 potential overestimates the Fe-ligand covalency for these two models. Their effective site spins lie between the intermediate spin ($S_1 = S_2 = 1$), and the high-spin ($S_1 = S_2 = 2$) AF-coupled limits. Also, the isomer shifts of the two iron sites in model III obtained from PW91 calculations (0.09 and 0.28 mm s⁻¹) are far from the experimental data (0.14 — 0.21 mm s⁻¹) [5,6]; although the pK_a mentioned above is around 7 in PW91, model III is not likely to represent the active site of MMOH-Q.

Net spin populations are the main indicator of the high spin or intermediate spin character of the Fe sites. In the ideal ionic limit, the net unpaired spin population is 4 for an Fe⁴⁺ (four unpaired d-electrons) $S = 2$ site. The absolute calculated net spins for all model clusters obtained from OPBE and OLYP methods are larger than 3 (see Tables 3 and 4), showing they are high spin Fe sites with substantial Fe-ligand covalency. The opposite signs for the spin densities of Fe1 and Fe2 for each model confirm the AF-spin coupling. OPBE and OLYP potentials predict reasonable quadrupole splittings for all four models (0.28 — 0.71 mm s⁻¹) which are close to the experimental data (0.53 — 0.68 mm s⁻¹) [5,6].

Model I yields the shortest Fe-Fe distance among the four models, and PW91 potential yields the shortest Fe-Fe distance (2.632 Å) for model I among the three methods. But this distance is still much longer than the EXAFS's prediction from measurement and analysis (2.46–2.52 Å) [7,8]. Further experimental and theoretical efforts are still needed to investigate the accuracy of such a short Fe-Fe distance for MMOH-Q.

The PW91 calculated isomer shifts for model I (0.31 and 0.18 mm s⁻¹) are very similar to our previous results obtained on a smaller model with different orientations of the water molecules and with fixed positions for the second-shell residues (0.36 and 0.19 mm s⁻¹) [29]. The newly PW91 calculated isomer shifts for model II (0.17 and 0.21 mm s⁻¹), are however, much different from the previous results (0.38 and 0.194 mm s⁻¹, see the Introduction section), and are very consistent with experiment (0.17 mm s⁻¹ for Mt [5], and 0.14 and 0.21 mm s⁻¹ for Mc protein [6]). The isomer shifts and quadrupole splittings of models I and II are very similar to each other in both OPBE and OLYP calculations. Since the electronic energies of model II in all three calculations are (by 2.87 — 9.13 kcal mol⁻¹) lower than the corresponding ones of

model I, we now think that model II is more likely than model I to represent the active site of MMOH-Q.

The EXAFS analysis of MMOH-Q shows a single short Fe-O bond of 1.77 Å, which leads the proposal of a possible $\text{Fe}_2^{4+}(\mu\text{-O})_2$ diamond core structure in Q with one short and one long Fe-($\mu\text{-O}$) bond [7]. In our current calculations, both models III and IV reveal a diamond core structure with shorter Fe1-O1 and Fe2-O2 bonds and longer Fe1-O2 and Fe2-O1 distances. The large value (28 units) of the pre-edge area for each Fe^{4+} site in Q observed by X-ray absorption experiment implies that the Fe^{4+} centers have a highly distorted geometry and are likely to have coordination number no greater than 5 [7]. If this is true, our model IV is the best to represent the distorted geometry and the 5–5 coordination numbers for both Fe sites. All models in OPBE and OLYP calculations present a long Fe2-O6 distance which is over 2.3 Å and can be considered as unbonded.

Our model IV is a completely new model where the His147 is protonated and H-bonding to the axial hydroxo. No similar models have been studied in the Fe-oxo systems. The energy of this model turns out to be the lowest among the four models in both OPBE and OLYP calculations. The energy difference (ΔE_0) between model IV and model II is 3.34 kcal mol⁻¹ in OPBE and 4.58 kcal mol⁻¹ in OLYP. On the contrary, model IV has the highest E_0 energy in PW91 calculations. However, this may be because PW91 potential overestimates the Fe-ligand covalency for models I-III. Model IV has the largest net spin populations (2.75 and 2.79) among the four models of PW91 calculations. They certainly reflect the two high-spin AF-coupled iron sites. Their calculated quadrupole splitting values (0.23 and 0.53 mm s⁻¹, see Table 5) are much smaller than those of models I-III. Although the predicted quadrupole splittings for the two Fe sites in model IV are rather different, considering the standard deviation of our calculations, they are still comparable with the experiment (0.53 — 0.68 mm s⁻¹) particularly for OPBE and OLYP. The calculated isomer shifts for model IV (0.11 — 0.22 mm s⁻¹) from the three calculating methods are also consistent with observed values (0.14 — 0.21 mm s⁻¹). Model IV is, therefore, also a promising candidate to represent the active site of MMOH-Q in common with model II. In the future, we plan also to examine the proton transfer barrier from the axial water molecule to His147.

5. Conclusions

Four structural models for MMOH-Q have been studied using broken symmetry density functional theory OPBE, OLYP, and PW91 functionals. Isomer shift parameters for OPBE and OLYP methods have been fitted. Standard deviations of the OPBE and OLYP calculated isomer shifts and quadrupole splittings for 34 sample Fe complexes are very similar to our previous PW91 calculations [29].

OPBE and OLYP methods yield very similar energetic, net spin population, and Mössbauer properties for the four MMOH-Q models (Figures 6-10). The diiron high-spin AF-coupled spin states are easily obtained by these two functionals. The PW91 potential seems to overestimate the Fe-ligand covalency if the structure is in the region of spin crossover.

By comparing the calculated properties of energies, pK_a 's, and Mössbauer isomer shifts and quadrupole splittings, model II (Figure 8) is likely to represent the active site of MMOH-Q. We have proposed a similar structure for the active site of RNR intermediate X (see Figure 4 of reference [30]). It is, therefore, very possible that MMOH-Q and RNR-X have the same central diiron structures, except that MMOH-Q has the $\text{Fe}^{4+}(\mu\text{-O})_2\text{Fe}^{2+}$ state, while RNR-X is in the $\text{Fe}^{3+}(\mu\text{-O})_2\text{Fe}^{2+}$ configuration. The two high-spin Fe sites in both molecules are AF-coupled.

Our current Model IV for MMOH-Q (Figure 10) has the lowest electronic energy among the four model clusters in both OPBE and OLYP calculations. The calculated isomer shifts and

quadrupole splittings of model IV are also comparable with the experiment. However, this structure may not exist depending on the $\text{H}_2\text{O} \rightarrow \text{His147}$ proton transfer barrier. Further calculations, including EXAFS spectra simulations [58], are underway to see whether the active site of MMOH-Q is exclusively represented by model II, model IV, or is a mixture of models II and IV, with some possible contribution from model I as well. Our calculations (for OPBE and OLYP especially) indicate that all of the models I, II, and IV are sufficiently low lying in energy to be potentially catalytically relevant.

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Figure 1. Possible active site structures for MMOH-Q proposed by (a) Siegbahn [11,12] and (b) Friesner and Lippard's group [16,17].

Figure 2.

Correlations between the OPBE calculated electron densities at Fe nuclei and the experimental isomer shifts for 17 $\text{Fe}^{2+,2.5+}$ (totally 31 Fe sites) and 19 $\text{Fe}^{2.5+,3+,3.5+,4+}$ (totally 30 Fe sites) sample complexes.

Figure 3.

Correlations between the OLYP calculated electron densities at Fe nuclei and the experimental isomer shifts for 17 Fe^{2+,2.5+} (totally 31 Fe sites) and 19 Fe^{2.5+,3+,3.5+,4+} (totally 30 Fe sites) sample complexes.

Figure 4.

Correlations between the calculated and the experimental isomer shifts for all 35 sample Fe complexes (totally 59 Fe sites) given in Tables 1 and 2 obtained from OPBE and OLYP potentials. The averaged values for the two $\text{Fe}^{2.5+}$ sites are used in the two fittings.

Figure 5. Correlation between the calculated and the experimental quadrupole splittings for all 35 sample Fe complexes (59 Fe sites in total) given in Tables 1 and 2 obtained from OPBE and OLYP calculations.

Figure 6. Model I of current study for the active site structure of MMOH intermediate Q. The names of the second and third shell H-bonding residues are labeled in the figure.

Figure 7.
The diiron core structure of our current model I. The whole structure of model I is shown in Figure 6.

Figure 8.
The diiron core structure of our current model II for the active site of MMOH-Q. The second and third shell residues shown in Figure 6 are also included in model II.

Figure 9. The diiron core structure of our model III for the active site of MMOH-Q. The second and third shell residues shown in Figure 6 are also included in model III.

Figure 10. Model IV for the diiron core structure of the active site of MMOH-Q. The second and third shell residues shown in Figure 6 are also included in model IV.

Table 1

Calculated and the Experimental Isomer shifts (δ_{cal} and $\delta_{4,2\text{K}}$) and Quadrupole Splittings ($\Delta E_{\text{Q(cal)}}$ and $\Delta E_{\text{Q(exp)}}$) (mm s^{-1}) of the Sample Fe^{2+} , and $\text{Fe}^{2.5+}$ Complexes Which Were Used in the Simple Linear Regression to Get the Linear Correlation between the Calculated (Using OPBE and OLYP Functionals) Electron Density on $\text{Fe}^{2+,2.5+}$ Nuclei ($\rho(0)$) and the Corresponding Experimental Isomer Shift Values at 4.2 K.^a

Complex	S _{total}	ox- state	experiment		OPBE calculated			OLYP calculated				
			δ _{4,2K}	ΔE _{Q(exp)}	ρ(0)	δ _{cal}	ΔE _{Q(cal)}	η _(cal)	ρ(0)	δ _{cal}	ΔE _{Q(cal)}	η _(cal)
FeF ₂ (FeF ₆) ⁴⁻	2	+2	1.48	2.85	11874.319	1.49	3.10	0.01	11874.344	1.49	3.67	0.21
FeCl ₄ ²⁻	2	+2	1.05	3.27	11875.238	1.19	3.49	0.09	11875.212	1.21	3.36	0.05
FeBr ₄ ²⁻	2	+2	1.12	3.23	11875.096	1.24	3.50	0.18	11875.163	1.22	3.34	0.21
Fe(NCS) ₄ ²⁻	2	+2	0.97	2.83	11875.691	1.05	3.18	0.01	11875.669	1.06	3.13	0.00
Fe ₂ (salmp) ₂ ²⁻	0	+2	1.11	2.24	11875.613	1.07	-1.58	0.17	11875.558	1.10	-1.75	0.15
Fe(H ₂ O) ₆	2	+2	1.11	2.24	11875.656	1.06	-1.60	0.17	11875.587	1.09	-1.75	0.14
Fe(phen) ₂ Cl ₂	2	+2	1.39	3.38	11874.446	1.45	3.37	0.03	11874.562	1.42	3.36	0.02
Fe(optda) ₂ Cl ₂	2	+2	1.05	3.15	11875.375	1.15	-2.70	0.43	11875.369	1.16	-2.90	0.47
Fe(Py) ₄ Cl ₂	2	+2	0.91	3.17	11875.201	1.21	3.39	0.20	11875.223	1.20	3.61	0.21
Fe(O ₂ C-CH ₃) ₄ (C ₅ H ₅ N) ₂	0	+2	1.16	3.14	11875.096	1.24	3.00	0.04	11875.089	1.25	3.18	0.04
	0	+2	1.12	3.05	11875.449	1.13	2.79	0.41	11875.512	1.11	2.77	0.39
Fe ₂ (μ-O ₂ C-CH ₃) ₄ (C ₅ H ₅ N) ₂												
Fe ₂ (μ-O ₂ C-CH ₃) ₂ (O ₂ C-CH ₃) ₂ (THF) ₂	4	+2	1.12	3.05	11875.435	1.13	2.83	0.35	11875.515	1.11	2.75	0.35
		+2	1.26	2.90	11875.402	1.14	2.87	0.08	11875.422	1.14	2.74	0.38
Fe ₂ (μ-O ₂ C-CH ₃) ₂ (O ₂ C-CH ₃) ₂ (NH ₂ CH ₂ CH ₃) ₂	0	+2	1.26	2.90	11875.394	1.14	2.74	0.21	11875.403	1.15	2.65	0.17
		+2	1.26	2.90	11875.225	1.20	2.88	0.03	11875.318	1.17	2.93	0.16
		+2	1.26	2.90	11875.252	1.19	2.80	0.09	11875.337	1.17	2.88	0.17
	4	+2	1.19	2.90	11875.400	1.14	2.67	0.34	11875.503	1.11	2.30	0.46
Fe ₂ (μ-OH) ₂ (μ-O ₂ C-CH ₃) ₂ (O ₂ C-CH ₃) ₂ (THF) ₂ (OH) ₂		+2	1.19	2.90	11875.446	1.13	2.64	0.42	11875.473	1.12	2.36	0.27
	0	+2	1.19	2.90	11875.405	1.14	3.20	0.03	11875.367	1.16	3.22	0.05
		+2	1.19	2.90	11875.409	1.14	3.21	0.04	11875.373	1.16	3.23	0.04
	4	+2	1.35	3.26	11874.826	1.32	2.93	0.31	11874.865	1.32	3.01	0.35
Fe ₂ BPMP(OPr) ₂ ⁺												
Fe(II)Fe(III)BPMP(OPr) ₂ ²⁺	1/2	+2	1.15	2.69	11875.742	1.03	2.96	0.13	11875.756	1.03	2.99	0.11
Fe ₂ (OH)(Oac) ₂ (Me ₃ TACN) ₂ ⁺	0	+2	1.16	2.83	11875.371	1.15	2.83	0.19	11875.321	1.17	2.91	0.27
Fe ₂ (salmp) ₂ ⁻	9/2	+2	1.16	2.83	11875.376	1.15	2.80	0.05	11875.322	1.17	2.87	0.14
		+2.5	0.83	1.08	11875.936	0.97	0.76	0.97	11875.938	0.97	0.85	0.91
		+2.5	0.83	1.08	11875.934	0.97	0.77	0.99	11875.936	0.97	0.86	0.94

^a See Table 1 of Ref. [29] for more notes and references, and the corresponding results for PW91 potential, $\rho(0)$ were calculated on the OPBE and OLYP COSMO optimized geometries.

Table 2

Calculated and Experimental Isomer shifts (δ_{cal} and $\delta_{4,2}$) and Quadrupole Splittings ($\Delta E_{\text{Q(cal)}}$ and $\Delta E_{\text{Q(exp)}}$) (mm s^{-1}) of a set of $\text{Fe}^{2.5+}$, Fe^{3+} , $\text{Fe}^{3.5+}$ and Fe^{4+} Complexes Which Were Used in the Simple Linear Regression to Get the Linear Correlation between the Calculated (Using OPBE and OLYP Functionals) Electron Density on $\text{Fe}^{2.5+}$, $3+, 3.5+, 4+$ Nuclei $\rho(0)$ and the Corresponding Experimental Isomer Shift Values at 4.2 K.^a

complex	S_{total}	ox- state	experiment		OPBE calculated				OLYP calculated			
			$\delta_{4,2}$	$\Delta E_{\text{Q(exp)}}$	$\rho(0)$	δ_{cal}	$\Delta E_{\text{Q(cal)}}$	$\eta_{\text{(cal)}}$	$\rho(0)$	δ_{cal}	$\Delta E_{\text{Q(cal)}}$	$\eta_{\text{(cal)}}$
$\text{Fe}_2(\text{salm})_2^-$	9/2	+2.5	0.83	1.08	11875.936	0.71	0.76	0.97	11875.938	0.71	0.85	0.91
$\text{Fe}(\text{bipy})_2\text{Cl}_2^+$	5/2	+2.5	0.83	1.08	11875.934	0.71	0.77	0.99	11875.936	0.71	0.86	0.94
		+3	0.54	0.24	11876.321	0.58	0.32	0.70	11876.294	0.60	0.29	0.56
FeF_6^{3-}	5/2	+3	0.61	0.00	11876.322	0.58	-0.02	0.40	11876.339	0.59	-0.02	0.48
FeCl_6^{3-}	5/2	+3	0.56	0.04	11875.862	0.73	0.05	0.81	11875.829	0.74	-0.05	0.24
FeCl_4^-	5/2	+3	0.36	0.00	11876.528	0.52	-0.03	0.58	11876.604	0.51	-0.02	0.36
$\text{Cl}_3\text{FeOFeCl}_3^{2-}$	0	+3	0.36	1.24	11876.961	0.39	-0.97	0.02	11877.045	0.37	-0.99	0.00
		+3	0.36	1.24	11876.957	0.39	-0.99	0.04	11877.046	0.37	-0.99	0.02
$\text{Fe}_2\text{O}(\text{OAc})_2(\text{HBpz})_2$	0	+3	0.55	1.60	11876.632	0.49	-1.47	0.56	11876.767	0.46	-1.49	0.56
		+3	0.55	1.60	11876.633	0.49	-1.45	0.57	11876.765	0.46	-1.46	0.57
$\text{Fe}_2(\text{OH})$	0	+3	0.50	0.25	11876.671	0.48	-0.34	0.25	11876.664	0.49	-0.34	0.15
$(\text{OAc})_2(\text{HBpz})_2^+$	0	+3	0.50	0.25	11876.673	0.48	-0.37	0.27	11876.663	0.49	-0.37	0.19
		+3	0.47	1.50	11876.710	0.46	-1.40	0.54	11876.822	0.44	-1.40	0.56
Fe_2O	0	+3	0.47	1.50	11876.703	0.47	-1.42	0.53	11876.821	0.44	-1.41	0.55
$(\text{OAc})_2(\text{Me}_3\text{TACN})_2^{2+}$	0	+3	0.41	1.80	11876.626	0.49	-1.38	0.49	11876.747	0.46	-1.42	0.50
		+3	0.41	1.80	11876.629	0.49	-1.36	0.40	11876.746	0.46	-1.41	0.43
$\text{Fe}_2(\text{salm})_2$	0	+3	0.56	0.88	11876.523	0.52	1.05	0.56	11876.476	0.55	1.05	0.53
		+3	0.56	0.88	11876.525	0.52	1.04	0.57	11876.478	0.55	1.05	0.53
$\text{Fe}(\text{II})\text{Fe}(\text{III})\text{BPMP}$	1/2	+3	0.50	0.50	11876.445	0.55	0.63	0.41	11876.417	0.56	0.68	0.44
$(\text{OPr})_2^{2+}$	0	+3	0.50	1.93	11876.817	0.43	1.70	0.95	11876.908	0.41	1.66	0.93
$\text{Fe}_2\text{O}_3(\text{6TLA})_2^{2+}$	0	+3	0.50	1.93	11876.815	0.43	1.70	0.95	11876.907	0.41	1.65	0.92
		+3	0.46	1.41	11876.752	0.45	1.31	0.89	11876.835	0.44	1.32	0.91
Fe_2O	0	+3	0.46	1.41	11876.752	0.45	1.32	0.91	11876.836	0.44	1.32	0.91
		+3	0.56	0.90	11876.301	0.59	1.26	0.83	11876.259	0.61	1.25	0.79
$\text{Fe}_2(\text{Cat})_4(\text{H}_2\text{O})_2^{2-}$	0	+3	0.56	0.90	11876.303	0.59	1.25	0.83	11876.257	0.61	1.25	0.80
$\text{Fe}_2\text{O}_3(5\text{-Et}_3\text{-TPA})_2^{3+}$	3/2	+3.5	0.14	0.49	11877.854	0.11	-0.38	0.31	11877.897	0.11	-0.42	0.35
		+3.5	0.14	0.49	11877.846	0.11	-0.38	0.23	11877.892	0.11	-0.42	0.31
$\text{Fe}(\text{OEC})\text{Cl}$	1	+4	0.22	2.99	11877.645	0.17	2.72	0.02	11877.604	0.20	2.66	0.03
$\text{Fe}(\text{OEC})\text{C}_6\text{H}_5$	1	+4	-0.08	3.72	11878.464	-0.08	3.06	0.07	11878.445	-0.06	2.93	0.07
$\text{FeCl}(\eta^4\text{-MAC}^*)^-$	2	+4	-0.04	-0.89	11877.978	0.07	-0.82	0.60	11877.930	0.10	-0.80	0.64

^a See Table 2 of Ref. [29] for more notes and references, and the corresponding results for PW91 potential, $\rho(0)$ were calculated on the OPBE and OLYP COSMO optimized geometries.

Table 3

Geometries ($\text{\AA}$), pK_a , Net-Spin Populations, Broken-Symmetry State Energies (E_{BS}), Spin-Projected Energies (E_0) (eV), Heisenberg J Values (cm^{-1}), Mössbauer Isomer Shifts (δ) (mm s^{-1}), Quadrupole Splittings (ΔE_Q) (mm s^{-1}), and η Values Calculated from OPBE Potential for the MMOH-Q Active Site Models I-IV (Figures 6-10), and Compared with Experimental Values.

	Model I	Model II	Model III	Model IV	Experiment
Geometry					
Fe I-Fe2	2.667	2.720	2.799	2.736	2.46–2.52 [7,8]
Fe I-O1	1.773	1.783	1.747	1.764	
Fe2-O1	1.773	1.791	1.866	1.817	
Fe I-O2	1.781	1.788	2.060	1.851	
Fe2-O2	1.794	1.803	1.696	1.761	
Fe I-O3		2.175	1.814	1.871	
Fe I-O4	1.884	1.929	1.935	1.900	
Fe I-N1	2.096	2.115	2.486	4.088	
Fe I-O5	2.104	2.085	2.025	2.004	
Fe2-O6	2.314	2.357	2.314	3.014	
Fe2-N2	2.085	2.111	2.144	2.092	
Fe2-O7	1.890	1.897	1.947	1.896	
Fe2-O8	2.058	2.029	2.081	1.976	
Fe I-O9	2.357				
O3...O9	3.563	2.601	2.759	3.348	
O3...O10	3.023	2.683		2.834	
O3...N1					
$pK_a(\text{H}_2\text{O})_{(\text{model II} \rightarrow \text{model III} + \text{H}^+)}$		14.78			
Net spin population Fe I	3.06	3.11	3.17	3.22	
Net spin population Fe2	-3.10	-3.15	-3.15	-3.23	
E_{BS}	-746.2979	-746.4150	-746.2685 ^a	-746.5772	
E_0	-746.4163	-746.5406	-746.3484 ^a	-746.6853	
Heisenberg J value	-239	-253	-161	-218	< -30 [5,6]
Mössbauer parameters					
δ_{FeI}	0.23	0.18	0.21	0.16	0.17(Mb) [5] 0.14(Mc) [6]
δ_{Fe2}	0.20	0.22	0.28	0.19	0.17(Mb) [5] 0.21(Mc) [6]
$\Delta E_Q(\text{FeI})$	-0.46	0.33	-0.38	0.70	0.53(Mb) [5] 0.55(Mc) [6]
$\Delta E_Q(\text{Fe2})$	-0.42	-0.33	-0.28	0.37	0.53(Mb) [5] 0.68(Mc) [6]
$\eta(\text{FeI})$	0.37	0.41	0.94	0.73	
$\eta(\text{Fe2})$	0.13	0.51	0.34	0.44	

^a Since model III has a different number of atoms (missing one H^+) compared to models I, II, and IV, energies E_{BS} and E_0 are not comparable. Instead, the $pK_a(\text{H}_2\text{O})$ relates model II to model III.

Table 4

Geometries ($\text{\AA}$), pK_a , Net-Spin Populations, Broken-Symmetry State Energies (E_{BS}), Spin-Projected Energies (E_0) (eV), Heisenberg J Values (cm^{-1}), Mössbauer Isomer Shifts (δ) (mm s^{-1}), Quadrupole Splittings (ΔE_Q) (mm s^{-1}), and η Values Calculated from OLYP Potential for the MMOH-Q Active Site Models I-IV (Figures 6-10).

	Model I	Model II	Model III	Model IV
Geometry				
Fe1-Fe2	2.703	2.746	2.838	2.756
Fe1-O1	1.790	1.799	1.760	1.777
Fe2-O1	1.791	1.807	1.883	1.833
Fe1-O2	1.794	1.807	2.103	1.866
Fe2-O2	1.817	1.821	1.708	1.775
Fe1-O3		2.221	1.832	1.889
Fe1-O4	1.894	1.943	1.947	1.914
Fe1-N1	2.106	2.131	2.482	4.121
Fe1-O5	2.136	2.112	2.047	2.026
Fe2-O6	2.339	2.394	2.366	3.093
Fe2-N2	2.104	2.125	2.151	2.110
Fe2-O7	1.897	1.911	1.969	1.911
Fe2-O8	2.081	2.049	2.103	1.991
Fe1-O9	2.455			
O3...O9	3.223	2.640	2.797	3.361
O3...O10	2.952	2.726		
O3...N1				2.868
$pK_a(\text{H}_2\text{O})_{(\text{model II} \rightarrow \text{model III} + \text{H}^+)}$		11.72		
Net spin population Fe1	3.05	3.10	3.14	3.17
Net spin population Fe2	-3.09	-3.14	-3.11	-3.19
E_{BS}	-719.5911	-719.7368	-719.7506	-719.9509
E_0	-719.6996	-719.8541	-719.8248	-720.0527
Heisenberg J value	-219	-237	-150	-205
Mössbauer parameters				
δ_{Fe1}	0.21	0.18	0.20	0.15
δ_{Fe2}	0.19	0.20	0.26	0.18
$\Delta E_Q(\text{Fe1})$	-0.44	0.30	0.35	0.71
$\Delta E_Q(\text{Fe2})$	-0.38	-0.29	-0.33	0.37
$\eta(\text{Fe1})$	0.24	0.36	0.95	0.71
$\eta(\text{Fe2})$	0.59	0.67	0.36	0.61

Table 5

Geometries ($\text{\AA}$), pK_a , Net-Spin Populations, Broken-Symmetry State Energies (E_{BS}), Spin-Projected Energies (E_0) (eV), Heisenberg J Values (cm^{-1}), Mössbauer Isomer Shifts (δ) (mm s^{-1}), Quadrupole Splittings (ΔE_Q) (mm s^{-1}), and η Values Calculated from PW91 Potential for the MMOH-Q Active Site Models I-IV (Figures 6-10).

	Model I	Model II	Model III	Model IV
Geometry				
Fe1-Fe2	2.632	2.689	2.716	2.661
Fe1-O1	1.765	1.777	1.784	1.758
Fe2-O1	1.767	1.780	1.776	1.839
Fe1-O2	1.809	1.807	1.830	1.832
Fe2-O2	1.778	1.800	1.808	1.755
Fe1-O3		1.959	1.800	1.840
Fe1-O4	1.911	1.983	1.979	1.907
Fe1-N1	2.040	2.053	2.124	3.573
Fe1-O5	2.076	1.971	2.058	2.005
Fe2-O6	2.113	2.061	2.051	2.278
Fe2-N2	2.034	2.066	2.079	2.050
Fe2-O7	1.913	1.945	1.988	1.918
Fe2-O8	2.012	1.920	1.946	1.979
Fe1-O9	2.203			
O3...O9	2.952	2.559	2.646	2.770
O3...O10	2.677	2.571		
O3...N1				2.773
$pK_a(\text{H}_2\text{O})_{(\text{model II} \rightarrow \text{model III} + \text{H}^+)}$		6.98		
Net spin population Fe1	2.60	1.96	1.92	2.75
Net spin population Fe2	-2.52	-1.96	-1.95	-2.79
E_{BS}	-744.9582	-745.2274	-745.3409	-744.9822
E_0	-745.1707	-745.5668	-745.7125	-745.1587
Heisenberg J value	-429	-684	-749	-356
Mössbauer parameters				
δ_{Fe1}	0.31	0.17	0.09	0.11
δ_{Fe2}	0.18	0.21	0.28	0.22
$\Delta E_Q(\text{Fe1})$	1.38	2.31	1.73	0.23
$\Delta E_Q(\text{Fe2})$	1.12	2.43	2.68	0.53
$\eta(\text{Fe1})$	0.49	0.07	0.22	0.83
$\eta(\text{Fe2})$	0.62	0.23	0.04	0.42