Reactivity of methane mono-oxygenase, insights from quantum mechanic studies on synthetic iron model complexes

Peter-Paul Knops-Gerrits\textsuperscript{a,d,*}, Pierre A. Jacobs\textsuperscript{b}, Atsushi Fukuoka\textsuperscript{c}, Masaru Ichikawa\textsuperscript{c}, Francesco Faglioni\textsuperscript{a}, William A. Goddard III\textsuperscript{a}

\textsuperscript{a} Material and Process Simulation Center, Beckman Institute (139-74), California Institute of Technology, Pasadena, CA 91125, USA
\textsuperscript{b} Center for Surface Science and Catalysis, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium
\textsuperscript{c} Catalysis Research Center, Hokkaido University, Sapporo 060, Hokkaido, Japan
\textsuperscript{d} Département de Chimie, Université Catholique de Louvain (UCL), Lavoisier, Place L. Pasteur no. 1, B-1348 Louvain-la-Neuve, Belgium

Abstract

Methane mono-oxygenase (MMO) and deoxyhemerythrin (DHr) are examples of di-iron enzymes that catalyze the dissociative and non-dissociative binding of molecular oxygen. To mimic the MMO active site with a finite cluster, we chose to study the binuclear heptapodate coordinated iron(III)-complexes of $\text{N,N,N}_0\text{N}_0\text{N}_0\text{N}$-tetakis(2-benzimidazolylmethyl)-2-hydroxy-1,3-diamino-propane (HPTB) and $\text{N,N,N}_0\text{N}_0\text{N}_0\text{N}$-tetakis(2-pyridylmethyl)-2-hydroxy-1,3-diamino-propane (HPTP). These have active sites of the form $[\text{Fe}_2(\text{HPTP}(\mu\text{-OH}))_4]^\text{C}(1)$ and $[\text{Fe}_2(\text{HPTP}(\mu\text{-OH}))_4]^\text{C}(2)$. Quantum mechanics structures are compared with the experimental data obtained from the EXAFS analysis. For the $\text{O}_2$ binding on the reduced active site, the $\text{m}_1:\text{h}_1\text{-O}_2$ mode seems the slightly more stable precursor to the $\text{O}=\text{Fe}–\text{O}–\text{Fe}=\text{O}$ bis-ferryl (re)active site. The nature of the ferryl groups are these of a reactive two center three electron bond. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Methane mono-oxygenase; Quantum mechanics; EXAFS analysis; Reactivity; Structure; Energetics; Orbital description

1. Introduction

Theoretical modeling of the structure and reactivity at enzyme active sites such as methane mono-oxygenase (MMO) is of intrinsic importance in a biological, chemical and industrial context [1–12]. Studies how the dioxygen cleavage [1–4] and methane activation [5–12] on these diiron enzyme core models takes place, has been studied with various methods. Yoshizawa et al. use density functional methods to study dioxygen binding with the extended Hückel method, an approximate molecular orbital method, which has also been used to study the reactivity with methane [1]. The $\mu\text{-}\text{Fe}–\text{Fe}=\text{O}_2$ binding mode is the most favorable binding mode of dioxygen on complexes on which one bridging ligand is removed [1]. The $\mu\text{-}\text{Fe}–\text{Fe}=\text{O}_2$ mode is most effective for electron transfer to the $\text{d}$-block orbitals. Methane mono-oxygenase and deoxyhemerythrin are examples of diiron enzymes that catalyze the dissociative and non-dissociative binding of molecular oxygen [4–12]. Dissociative binding of oxygen via a peroxo intermediate to a diamond core structure [4] leads to a reactive species active in the oxidation of alkanes [4,5]. Methane mono-oxygenase has a binuclear active site in which two histidines and four glutarates are present. Both iron ions are coordinated by a histidine, an oxygen from a bridging carboxylate and a...
μ-oxo bridge [5]. Non-dissociative binding of oxygen via a side-on peroxo intermediate such as in the active site of deoxyhemerythrin does not allow the splitting and allows binding/release of oxygen as a function of the physiological conditions [10]. Such active sites related to oxo- and hydroxo-bridged di- or poly-iron cores in biological systems [8,9] have been recently subject to extensive theoretical modeling [1–3,13–16]. Siegbahn et al. showed that model systems of MMO with only simple aqua and carboxylato ligands having intermediates with plausible energies and structures and a low-energy transition for the C–H abstraction [3]. In these gas-phase calculations, the dielectric constant of the enzyme is low (ε=4) compared to this of water (ε=80) [3]. For solvation of model compounds in water such effects are not negligible. For the Fe(IV)-complexes 5- or 6-coordination behave as well, for Fe(III)-complexes such as Fe(OH)3(OH)3 5-coordination is favored, whereas for Fe(OH)26 6-coordination is observed. For Fe(II)-complexes 4-coordination occurs and extra water molecules are present in a second shell. Regarding methane activation Crabtree [2] reviewed the recent data. According to Siegbahn et al., [3] the most significant structure is the FeIII–O–Fe V = O oxo structure. The ground state of this structure is 11 Å and the iron spins are 4.00 and 2.94, the spin on the bridging oxygen is 0.76 and on the oxo ligand is as high as 1.13. The oxide cations of iron have a high spin ground state and adjacent low spin excited state and posses the same bonding patterns as the oxygen molecule [17]. Good bonding of the low spin state, leads to a spin cross-over along the reaction coordinate and opens a low-energy TSR (two state reactivity) path for hydroxylation. The mimicking of such enzymes by immobilization of the model complexes [18–23] in the voids of clays or mesoporous silica–alumina is our interest [24–28]. The characterization of [Fe2(HPTB)(μ-OH)(NO3)2](ClO4)2 (1) as seen in Fig. 1 and [Fe2(HPTB)(μ-OH)(NO3)2](NO3)2 (2) with EXAFS–XANES, spectroscopic tools and a complimentary theoretical quantum structure analysis is investigated here.

2. Experimental

2.1. Synthesis

2.1.1. [Fe2(HPTB)(OH)(NO3)2](NO3)2

_N,N,N’,N’-tetrakis(2-benzimidazolylmethyl)-2-hydroxy-1,3-diaminopropane (HPTB) is prepared [4,18,24]. To an ethanol solution of Fe(NO3)3·6H2O (0.31 g) the HPTB (0.30 g) is added. The precipitated iron complex is collected.

2.1.2. [Fe2(HPTP)(OH)(NO3)2](ClO4)2

_N,N,N’,N’-tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropane H(HPTP) as perchlorate is prepared from p-chloropicoline and 2-hydroxy-1,3-diaminopropane after [10]. As in the previous synthesis, Fe(NO3)3·6H2O (0.31 g) and H(HPTP)(ClO4)2 (0.28 g) are solved in ethanol. The complex is washed with acetonitrile/diethylether and recrystallized in diethylether.

2.2. EXAFS–XANES characterization

EXAFS–XANES X-ray absorption spectra were conducted on beamline BL-7C, under ring conditions of 2.5 GeV, 200–400 mA in the photon factory of the National Laboratory of High Energy Physics (KEK-PF). The radiation was mono-chromatized using a Si(111) double crystal mono-chromator. Data were measured in transmission mode with N2-filled ionization chamber of 17 cm and a N2/Ar (85/15)-filled ionization chamber of 31 cm to k = 10 Å−1 for XAS and to k = 16 Å−1 for EXAFS. Sample preparation involves pressing BN pellets of a diameter of 6.4 mm with an iron concentration of 10 wt.% for pure samples. Care should be taken in the analysis of highly diluted samples (in HMS or other supported samples) because of the X-ray absorbance of the sample. Energies were calibrated using an internal Fe foil standard, assigning the Fe K-edge to

![Figure 1. Structure of [Fe2(HPTP)(μ-OH)(NO3)2](NO3)2.](image-url)
7111 eV. In the figures the \( \chi(k) \) extraction, the Fourier conversion and the McKale spherical wave curve fitting data are shown. EXAFS–XANES file analysis and curve fitting is performed with TECHNOS software, a curve fitting was performed to evaluate the contributions of Fe, C, N and O atoms and their inter-atomic distances.

2.3. Computational analysis

The ab initio calculations used involve full geometry optimization of the clusters with density functional theory (DFT) as implemented in Jaguar [15] (Jaguar 3.0, Schrodinger Inc., Portland, Oregon, 1997) at the B3LYP method level (Becke3 hybridization functionals, Slater/Becke88 non-local exchange and Li, Yang, Parr local and non-local correlation corrections to the local potential energy functionals of Vosko, Wilk and Nusair) using the Los Alamos effective core potential and valence double Zeta for iron (LACVP basis sets). Calculations are carried out on an Origin2000 (16 MIPS R10000 (IP27) CPU’s, 195 MHz w/4 MB secondary cache each, with an IRIX 6.4 S2MP + OCTANE operating system).

The molecular mechanics calculations involve a new molecular mechanics force field, the Universal force field (UFF) of Rappé et al. and co-workers [13,14]. The force field parameters are estimated using general rules based only on the element, its hybridization and its connectivity. The force field functional forms, parameters, and generating formulas for the full periodic table have been published [13]. For charge equilibration used in molecular dynamics simulations, the charges in the complexes are determined [16]. This helps to readjust charges based on geometry and experimental atomic properties.

The initial structures were energy minimized using a suitable set of parameters that are appended to the UFF. These better describe the respective coordination around iron in the model compounds. The quantum mechanical study was performed at the ab initio level starting from a UFF optimized structure. UFF optimization uses a Newton–Raphson minimization scheme with a norm of the gradient convergence criteria of 1 \( \times \) 10\(^{-10} \) kcal/mol/Å and is verified by the absence of negative Eigen values in the force constant matrix. In order to accommodate the iron in its five coordinate form, as predicted by the EXAFS data, after UFF optimization, a QM calculation is performed. Atom types for iron and other transition metals in the UFF is given with its symbol, hybridization and valence state. UFF contains 126 atom types, the force constants are generated using Badgers rules as described, the van der Waals parameters are computed based on a Lennard–Jones type potential [13,14].

2.4. Spectroscopic characterization

Diffuse reflectance spectroscopy (DRS) spectra are recorded on a Varian Cary 5 photometer. Mössbauer spectra are recorded on a horizontal constant acceleration drive in transmission geometry with a 150 mCi \( ^{57} \) Co (Rh) source. The 14.4 keV Mössbauer transition of the lowest excited level of \( ^{57} \) Fe to its ground state \((E_e - E_g = 14.4 \text{ keV}, t_{1/2} = 98 \text{ ns})\) and natural linewidth \((4.7 \times 10^{-9} \text{ eV})\) is fed by the decay of \( ^{57} \) Co \((t_{1/2} = 270 \text{ days})\). MS is a microscopic, nuclear technique, based on the recoilless emission and absorption of \( \gamma \)-rays by a ‘source’ and an ‘absorber’. MS determines the recoilless fraction (or f-fraction) and the hyperfine parameters (isomeric shift, quadrupolar splitting and the magnetic splitting) of the MS-nucleus. SQUID spectra are recorded on an MPMS SQUID spectrometer of quantum design. EPR spectra are recorded on a Varian Varian E-line spectrometer, with a liquid He cooling attachment.

3. Results and discussion

3.1. EXAFS spectroscopy

The Fe K-edge EXAFS–XANES analysis gives direct information of the coordination environment of the complexes as salts. The Fe K-edge XAS on \([\text{Fe}_2(\text{HPTP})(\mu-\text{OH})(\text{NO}_3)_2][\text{NO}_3]\) and \([\text{Fe}_2(\text{HPTB})(\mu-\text{OH})(\text{NO}_3)]([\text{NO}_3])_2\) show pre-edge features indicative of a distorted five coordinated iron with a sixth Fe-ligand, the edge-shifts of 13.1 and 15.5 eV are characteristic for its high spin ferric form. The EXAFS region is informative for a number of parameters including coordination number (CN), inter-atomic distance (R), correction of threshold energy (\(\Delta E\)), and difference of the Debye–Waller factor from reference compounds (\(\Delta \sigma^2\)) derived by the analysis of EXAFS spectra. As seen in
Table 1
Fourier transform range Fe K-edge EXAFS analysis

<table>
<thead>
<tr>
<th>Samples</th>
<th>Shell</th>
<th>$R^a (\text{Å})$</th>
<th>$\text{CN}^b$</th>
<th>$\sigma^c$</th>
<th>$\Delta E_{\text{fit}}^d (\text{eV})$</th>
<th>Residual$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPTB/Fe</td>
<td>Fe–Fe</td>
<td>3.233</td>
<td>1</td>
<td>0.062</td>
<td>$-1.34$</td>
<td>7.76</td>
</tr>
<tr>
<td></td>
<td>Fe–O</td>
<td>1.933</td>
<td>1</td>
<td>0.025</td>
<td>$-7.33$</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>Fe–O</td>
<td>1.919</td>
<td>2</td>
<td>0.128</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe–N</td>
<td>2.105</td>
<td>2</td>
<td>0.048</td>
<td>14.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe–N</td>
<td>2.327</td>
<td>1</td>
<td>0.016</td>
<td>9.92</td>
<td></td>
</tr>
<tr>
<td>HPTB/Fe/HMS$^f$</td>
<td>Fe–Fe</td>
<td>3.347</td>
<td>1$^f$</td>
<td>0.049</td>
<td>7.05</td>
<td>11.96</td>
</tr>
<tr>
<td></td>
<td>Fe–N/O</td>
<td>2.25</td>
<td>5</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>HPTP/Fe</td>
<td>Fe–Fe</td>
<td>3.020</td>
<td>1</td>
<td>0.130</td>
<td>$-0.99$</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>Fe–O</td>
<td>1.929</td>
<td>1</td>
<td>0.055</td>
<td>$-9.65$</td>
<td>9.47</td>
</tr>
<tr>
<td></td>
<td>Fe–O</td>
<td>1.936</td>
<td>1</td>
<td>0.120</td>
<td>16.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe–N</td>
<td>2.185</td>
<td>2</td>
<td>0.073</td>
<td>18.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe–N</td>
<td>2.384</td>
<td>1</td>
<td>0.115</td>
<td>10.74</td>
<td></td>
</tr>
<tr>
<td>HPTP/Fe/HMS$^f$</td>
<td>Fe–Fe</td>
<td>3.216</td>
<td>1$^f$</td>
<td>0.119</td>
<td>2.10</td>
<td>11.46</td>
</tr>
<tr>
<td></td>
<td>Fe–N/O</td>
<td>2.25</td>
<td>3</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe–O</td>
<td>1.99</td>
<td>2</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Inter-atomic distance.
$^b$ Coordination number.
$^c$ Debye–Waller factor.
$^d$ Energy (eV).
$^e$ $R$ factor (%).
$^f$ Hexagonal mesoporous silica.

Using the TECHNOS software. Sometimes a slightly larger value (up to 1.25) may reflect iron aggregation and silicon/aluminum coordination.

Table 1, the Fe K-edge EXAFS indicates that the [Fe$_2$(HPTB)(μ-OH)]$^{4+}$ gives a coordination number (CN) of 1 for the Fe–Fe bonding and an Fe–Fe distance of 3.020 Å, the [Fe$_2$(HPTB)(μ-OH)]$^{4+}$ gives a CN of 1 for the Fe–Fe bonding and an Fe–Fe distance of 3.233 Å in accordance with the crystallographic data [18–20]. For complexes with bulkier HPTB ligands μ-OH species are seen in the Mössbauer spectra due to longer Fe–Fe distances, with less bulky HPTP ligands, two species with longer and shorter Fe–Fe distances are seen, in Table 2, closer to μ-OH and μ-O coordination, respectively.

Compared with our computations shorter Fe–OH inter-atomic distances are seen of 1.92–1.99 Å with EXAFS. The Fe–OH distance might be slightly shorter then this observed in the crystallographic structure [5]. The two coordinated benzimidazole groups in HPTB give Fe–N bonds of 2.105 Å and a longer Fe–N inter-atomic distance of 2.327 Å for the amine N. The two coordinated pyridine groups in HPTP give Fe–N bonds of 2.185 Å and a longer Fe–N inter-atomic distance of 2.384 Å for the amine N. The large Debye–Waller factor arises from the wide range of Fe-ligand bond lengths in a sample consisting of several different species.

Table 2
Mössbauer spectral data for enzymes and their mimics$^a$

<table>
<thead>
<tr>
<th>Species</th>
<th>$\delta$ (mm/s)$^b$</th>
<th>$\Delta$ (mm/s)</th>
<th>Relative intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Fe(Sq$^b$)(OH)(4-Me-py)]$_2$</td>
<td>0.33</td>
<td>0.55</td>
<td>100</td>
</tr>
<tr>
<td>[(N-base)PcFe]$_2$O</td>
<td>0.17–0.20</td>
<td>1.6–1.76</td>
<td>100</td>
</tr>
<tr>
<td>[Fe$_2$O(Oac)$_2$(HBPz$_3$)$_2$]</td>
<td>0.52</td>
<td>1.60</td>
<td>100</td>
</tr>
<tr>
<td>[Fe$_2$O(Oac)$_2$(bpy)$_2$(Cl)$_2$]</td>
<td>0.37</td>
<td>1.80</td>
<td>100</td>
</tr>
<tr>
<td>Methemerythrin</td>
<td>0.46</td>
<td>1.57</td>
<td>100</td>
</tr>
<tr>
<td>Oxyhemerythrin</td>
<td>0.52</td>
<td>1.92</td>
<td>50</td>
</tr>
<tr>
<td>Oxyhemerythrin</td>
<td>0.48</td>
<td>1.00</td>
<td>50</td>
</tr>
<tr>
<td>[Fe$_2$(N-Et-HPTB)(OBz)]</td>
<td>1.07</td>
<td>3.13</td>
<td>100</td>
</tr>
<tr>
<td><a href="OBz">Fe$_2$(N-Et-HPTB)</a>]</td>
<td>0.52</td>
<td>0.72</td>
<td>100</td>
</tr>
<tr>
<td>[Fe$_2$Hbab$_2$(C-Im)$_2$]</td>
<td>0.52</td>
<td>3.26</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$ From [4–12,18–23].
$^b$ Squarate, C$_4$O$_4^{2−}$.
$^c$ Values are expressed relative to metallic Fe at 300 K.
3.2. Computational analysis

3.2.1. Modeling the structures

The \([\text{Fe}_2(\text{HPTP})(\mu-\text{OH})]^{4+}\) (1), and \([\text{Fe}_2(\text{HPTB})(\mu-\text{OH})]^{4+}\) (2) complexes are used in catalysis. These are models that have a structure that combines all the features of the ligand and the iron active site. The \([\text{Fe}_2(\text{HPTM})(\mu-\text{OH})]^{4+}\) complex was used in the calculations and an iminomethyl group is positioned where a pyridine or a benzimidazole occur in the actual ligands as seen in Fig. 1. Both ferric \([\text{Fe}_2(\text{HPTM})(\mu-\text{OH})]^{4+}\) and ferrous \([\text{Fe}_2(\text{HPTM})(\mu-\text{OH})]^{3+}\) cores are then optimized with quantum mechanics (QM). Compared with our EXAFS data the Fe–Fe distance of 3.135 Å obtained on the \([\text{Fe}_2(\text{HPTM})(\mu-\text{OH})]^{4+}\) model complexes is intermediate to this of the experimentally studied complexes. The QM optimized structure of \([\text{Fe}_2(\text{HPTM})(\mu-\text{OH})]^{4+}\) is reacted with oxygen in an acidic medium to give intermediates P and Q as shown in Fig. 3. Their interactions with CH₄ will be discussed elsewhere [22].

The charge of the \([\text{Fe}_2(\text{HPTM})(\mu-\text{OH})]^{4+}\) cluster is +4 or +2, depending on the simulation of a Fe(III) or an Fe(II) active site and the energy levels of different multiplicity are studied. The bond lengths and angles obtained by UFF are the approximate starting point. The relative energies of some important catalytic reactants were analyzed, as are the effects of their solvation again obtained by QM calculations. The UFF bond stretch types, angle bending types and Lennard–Jones 12-6 parameters for the model complexes have been determined as seen in Tables 3–6. Due to the change of a ferrous to a ferric type, the decrease in the bond length and the increase in the force constant for the Fe–X bond are seen and some changes in the angle bending parameters are observed. Only a slight increase is seen for the angles of the N₂ from 111.2 to 111.3°, these for the O–R increase from the standard 110–128.0 and 126.4° in the diferric core. Also, a decrease of the O–R Fe O–R angle can be seen from 90°.

### Table 3
Universal force field (UFF) bond stretch types

<table>
<thead>
<tr>
<th>R_{ij}</th>
<th>K_{ij}</th>
<th>R_{ij}n</th>
<th>K_{ij}n</th>
<th>R_{ij}n - R_{ij}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃ + 2N₂</td>
<td>2.080000</td>
<td>549.4361</td>
<td>2.0367</td>
<td>585.2129</td>
</tr>
<tr>
<td>Fe₃ + 2O–R</td>
<td>2.050000</td>
<td>500.5698</td>
<td>1.9666</td>
<td>566.9955</td>
</tr>
<tr>
<td>O–R–H</td>
<td>1.034000</td>
<td>984.3983</td>
<td>1.0121</td>
<td>1049.6934</td>
</tr>
<tr>
<td>O–R–C₃</td>
<td>1.437000</td>
<td>984.1528</td>
<td>1.4156</td>
<td>1029.5547</td>
</tr>
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</table>

### Table 4
Universal force field (UFF) angle bending types

<table>
<thead>
<tr>
<th>θ_{ijk} (bond angle)</th>
<th>p (Period cosine)</th>
</tr>
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<tbody>
<tr>
<td>Fe₃ + 2N₂–H</td>
<td>85.1612</td>
</tr>
<tr>
<td>Fe₃ + 2N₂–C₂</td>
<td>180.2790</td>
</tr>
<tr>
<td>Fe₃ + 2O–R–H</td>
<td>179.5816</td>
</tr>
<tr>
<td>Fe₃ + 2O–R–C₃</td>
<td>176.1039</td>
</tr>
<tr>
<td>N₂–Fe₃–C₂N₂</td>
<td>159.8253</td>
</tr>
</tbody>
</table>

### Table 5
Universal force field (UFF) Lennard–Jones 12-6 parameters

<table>
<thead>
<tr>
<th>X_{ij}</th>
<th>D_{ij}</th>
<th>Scale ζ</th>
</tr>
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<tbody>
<tr>
<td>2.88600</td>
<td>0.04400</td>
<td>12.0000</td>
</tr>
<tr>
<td>3.85100</td>
<td>0.10500</td>
<td>12.7300</td>
</tr>
<tr>
<td>3.66000</td>
<td>0.06900</td>
<td>13.4070</td>
</tr>
<tr>
<td>3.50000</td>
<td>0.06000</td>
<td>14.0850</td>
</tr>
<tr>
<td>2.91200</td>
<td>0.01300</td>
<td>12.0000</td>
</tr>
</tbody>
</table>

\(^{a}\) Van der Waals bond length in Å.

\(^{b}\) Well depth in kcal/mol.

\(^{c}\) The scale factor ζ shows that a Lennard–Jones exponential 6 form is chosen.
Table 6
Geometries of the complexes from experiment and force fields a

<table>
<thead>
<tr>
<th></th>
<th>Experiment</th>
<th>MSX</th>
<th>UFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe–O/Fe–N</td>
<td>1.946</td>
<td>1.900</td>
<td>1.852/2.080</td>
</tr>
<tr>
<td>Fe–O</td>
<td>2.116</td>
<td>2.099</td>
<td>2.135/2.050</td>
</tr>
<tr>
<td>Fe–O–Fe</td>
<td>119.7</td>
<td>123.4</td>
<td>91.6/109.471</td>
</tr>
<tr>
<td>O–Fe–O</td>
<td>102.5</td>
<td>102.8</td>
<td>80.5/74.5</td>
</tr>
</tbody>
</table>

a Experimental data from XRD, EXAFS and IR correlations [9] and force fields: material simulation X (MSX) and universal force field (UFF) after [13,14].

The complex has C2h symmetry and both ferrous iron ions can occur either in a high-spin quintet state, and intermediate-spin triplet state or a low-spin singlet state, when these states couple, we obtain a non-uplet state if the two iron ions are high-spin,

\[ N (dxz)^2(dxy)(dyz)(dz^2)(dx^2 - y^2) \]

or singlet S, when the two iron ions are in low-spin form.

\[ S (dxz)^2(dxy)^2(dy_z)^2 \]

When both ferric iron ions can occur either in a high-spin sextet state, and intermediate-spin quartet state or a low-spin doublet state, when these states couple we obtain the undecuplet state if the two iron ions are high-spin,

\[ U (dxz)(dxy)(dyz)(dz^2)(dx^2 - y^2) \]

the septet state, SP if the two iron ions are intermediate-spin,

\[ SP (dxz)^2(dxy)(dyz)(dz^2) \]

Fig. 2. QM optimized structures of [Fe^{III}_{2}(HPTM)(\mu-OH)]^{2+} of [Fe^{III}_{2}(HPTM)(\mu-O_2)]^{3+} of [(Fe^{IV}=O)_{2}(HPTM)]^{3+} and of [Fe^{III}_{2}(\mu-O_2)(HPTM)(\mu-O_2)]^{3+}. The interatomic FeO, Fe–Fe, O–O and O–H distances (Å) and FeOFe, FeOO, FeOH and OFeO angles (°) are given in and under the figure respectively.
or triplet T, when the two iron ions are in low-spin form.

\[ T = (d\pi)^2(d\pi)^2(d\pi)^2 \]

In the ferric case (Fe\textsuperscript{III}), the septet (SP) and the undecuplet (U) are its ground state and low lying exited states. The equatorial (trans) and axial (cis) effect of the N atoms with respect to the bridging \( \mu \)-oxo groups dictate their bond lengths that are 2.03–2.04, 2.31 and 1.95–1.99 Å, respectively. The bond distances of 1.95 and 1.99 Å obtained from QM seen in the direction of the equatorial unprotonated and protonated O groups compared to the calculations, are accompanied by Fe–O–Fe angles close to the 90–93° and to a O–Fe–O bite angle of 74.5°. The Fe coordinated to the axial N group has long bond distances of 2.31 Å seen in the QM calculations. The bond length of Fe to the equatorial N groups show short bond distances of 2.03–2.04 Å seen in the QM calculations, the N–Fe–N bite angle varies around values of 110.0° in QM. In QM, the order of the angles is such that Fe–O–R > Fe–N–R > Fe–O–Fe–N–Fe–N > O–Fe–O, in particular the first two values are much higher in QM compared to UFF and the last two values are much lower in QM compared to UFF. In UFF the N–Fe–N angles are larger than the Fe–O–Fe angles. Such QM data are used to change UFF parameters as seen in Tables 3–6 to better position the ligands (HPTB, HPTP) and allow to study larger systems with QM-parametrized modified UFF parameters.

### 3.2.2. Modeling the oxygen activation

Geometrical and electronic properties affect the relative catalytic properties, such as the hydrogen bond abstraction energies of the binuclear cores of these iron complexes. In the QM optimized structure, the iron core has two \( \mu \)-oxo and six terminating nitrogen atoms, the B3LYP calculations will now be performed on all the reaction intermediates. In these structures differences between the equatorial and axial nitrogen atoms are seen. In aqueous reactions, in acidic media, the bridging by the deprotonated ligand alcohol group remains strong, whereas the bridging (\( \mu \)-OH)
can be protonated and removed as water and lead to the binding of molecular oxygen and the consequent transformation of this intermediate into an oxidized bis-ferryl form [26]. During this transformation, the oxygen molecule is transformed in a peroxo (O$_2^{2-}$) group (intermediate P) with the formal change of the charges of iron from +2 to +3.

This leads to a diamagnetic singlet state

S (d$_{xz}^2$(d$_{xy}$)$^2$)(d$_{yz}$)(O$_2\pi$)(d$_{z^2}$)(O$_2\sigma$)

here the d$_{yz}$ orbital is coupled to the two orthogonal three electron pi-system of the O$_2$ ligands.

The alternative is a paramagnetic quintet state

Q (d$_{x^2-y^2}$)(d$_{xz}^2$(d$_{xy}$)(d$_{y^2}$)(O$_2\pi$)(d$_{z^2}$)(O$_2\sigma$)

The two double ferryl type bonds can become stronger by transfer of the sigma bonding electrons between the two oxygen atoms to their anti-bonding orbitals and the pairing of these with an extra electron from the iron ion. In a consequent step the peroxo (O$_2^{2-}$) group is transformed into two ferryl (O$_2^{-}$) bound groups (intermediate Q), with the formal change of the charges of iron from +3 to +4. In an alternative step, the peroxo (O$_2^{2-}$) group of the complex can also transform to yield two bridging ($\mu$-O) oxo groups, additional to the ligand bound O, which remains coordinated.

For the diferric complex and the diferrous complex, the high spin state seems to be the ground state. From these calculations it is clear that superexchange coupling for these complexes is fairly small, this agrees with the experimental observation of a $J$ value of 12 cm$^{-1}$ for the diferrous OH bridged model compound [18–20].

The geometrical implications on these reactions are probed with QM B3LYP analysis and the results are shown in Figs. 2–4 and Table 6. The oxygen bond length of 1.21 increases to 1.31 Å on the model compound and is smaller than the 1.49 Å distance in hydrogen peroxide, consequently this bond is broken in the model compound. The Fe···Fe distances in the model compound [Fe$_2$(HPTM)(H$_2$)]$^{3+}$ of 3.14 Å increases to 3.43 Å in intermediate P and 3.63 Å in intermediate Q. Upon transformation into the bis ($\mu$-O$_2^{2-}$) oxo bridged dimer, this distance decreases to 2.65 Å. The energy of the different intermediates seem fairly comparable with the peroxo form or intermediate P, being slightly more stable than the ferryl form or intermediate Q by about 18.9 kcal/mol. The formation of the bis ($\mu$-O$_2^{2-}$) oxo seems unfavorable since it is 44.4 kcal/mol higher in energy. The charges have been analyzed by Qeq and electrostatic potential QCESP charge fitting as seen in Table 7. Solvation proves higher in complexes with
increased charge transfer, i.e. Fe(IV)-complexes are better stabilized by solvation than Fe(III)-complexes. The solvation calculations given in Table 8 are important to obtain good quantitative data of the overall reactivity of the system. The solvation calculations use a Poisson–Boltzmann type solvation assuming a dielectric constant of water (ε = 80.37) and a known probe radius (1.40 Å). As the organic part of the complex renders the core hydrophobic and most of the distortions in the d4 bis-ferryl μ-oxo-bridged site with the change to 1/2Fe=O to 1/2Fe=O increases the reactivity of the system. The solvation calculations given in Table 8 are important to obtain good quantitative data of the overall reactivity of the system. The solvation calculations use a Poisson–Boltzmann type solvation assuming a dielectric constant of water (ε = 80.37) and a known probe radius (1.40 Å). As the organic part of the complex renders the core hydrophobic and most of the distortions in the d4 bis-ferryl μ-oxo-bridged site with the change to 1/2Fe=O to 1/2Fe=O increases the reactivity of the system. The solvation calculations give in Table 8 are important to obtain good quantitative data of the overall reactivity of the system.

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3.2.3. Description of the orbitals involved in the reactive site

The frontier orbitals for the ground state iron core with a μ-carboxy back-bone and a μ-oxo group or μ-hydroxo group have significant d-character. The unpaired valence electrons on the individual iron cores can also be localized primarily in the d-orbitals. The unpaired electron for the oxidized and the non-plet for the reduced resting state model has the lowest energy. For the intermediate Q, the orbitals that participate at the active site were studied. In Fig. 5, the most relevant binding orbitals are seen, the Fe–O covalent σ-bond and the C=O σ-bond as well as the σ- and π-bonds of the ferryl Fe=O in the plane of the Fe–O–Fe bridge can be seen. The active bis-ferryl μ-oxo-bridged site shows eight unpaired electrons. The localization of these free d-electrons occurs partially on the iron (FeIV) like with a spin density of 2.6 to 2.8 and partially on the oxygen (spin density around 0.80 and 0.85). Siegbahn [3] found that the spin on iron varies from 4.00 on FeIII, 3.75 on FeII and 3.2 on FeIV. As implied by the spin density analysis, the nature of the ferryl is closer to a two atom three-electron bond as to a double bond involving four electrons. During the transformation, the oxygen molecule is transformed in a peroxo (O22−) group (intermediate P) and a bis-ferryl μ-oxo-bridged site with the change of the formal charges of iron from +2 to +3 and +4. Siegbahn [3] found that due to the large Jahn–Teller distortions in the d4 bis-μ-oxo species the Fe–O–Fe bridges are highly asymmetric, allowing the system to open up easily to a key FeIII –O–FeIV=O intermediate that can react with methane via a low-energy transition state. This intermediate FeIII –O–FeIV–O0 with radical character at the terminal oxo-group is in accordance with our spin localization. After the H abstraction from methane, the methyl radical recombines rapidly with the Fe center via a weak Fe–CH3 bond in his model and with the loss of methanol FeIII–O–FeIII is formed which can be reduced to the Fe(II, II) form.

3.3. Diffuse reflectance spectroscopic (DRS) analysis

The oxygen adducts of the binuclear Fe(II)-complexes are formally analogous to the peroxide adducts of the Fe(III)-complexes e.g. oxy-hemerythr

Table 7
Charges of the [Fe2(O=)2(O)L]3+ complex from QC and Qeq*^a

<table>
<thead>
<tr>
<th>Species</th>
<th>QCESP</th>
<th>Qeq*N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>+1.158, +1.188</td>
<td>+1.104, +1.116</td>
</tr>
<tr>
<td>O−</td>
<td>−0.765</td>
<td>−0.443</td>
</tr>
<tr>
<td>Fe</td>
<td>−0.290, −0.301</td>
<td>−0.425</td>
</tr>
<tr>
<td>N</td>
<td>+0.420, +0.050</td>
<td>+0.191, +0.050</td>
</tr>
<tr>
<td>N</td>
<td>−0.704, −0.621</td>
<td>−0.285, −0.275</td>
</tr>
<tr>
<td>C</td>
<td>+0.466, +0.363</td>
<td>−0.419, −0.409</td>
</tr>
<tr>
<td>C−(O−)</td>
<td>+0.364, +0.321</td>
<td>+0.110</td>
</tr>
</tbody>
</table>

*Electronegativities are XFe = 3.5, XO = 8.741, XC = 5.343, XN = 6.899, XH = 4.528. Hardness and radius for Fe are modified to 1/2Fe=O = 3.124 and RFe = 1.293, respectively.

Table 8
Relative solvation energies obtained by QM calculations for active intermediates of the complexes in which (O)L = HPTM and M = model C=N

<table>
<thead>
<tr>
<th>Species, charge</th>
<th>Absolute energy (hartrees)</th>
<th>M</th>
<th>Spin</th>
<th>Energy difference (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Fe2(OH)(O)L]2+</td>
<td>−1158.405161</td>
<td>9</td>
<td>4</td>
<td>155</td>
</tr>
<tr>
<td>[Fe2(OH2)(O)L]3+</td>
<td>−1234.245871</td>
<td>7</td>
<td>3</td>
<td>335</td>
</tr>
<tr>
<td>[Fe2(O=)(O)L]3+</td>
<td>−1232.996704</td>
<td>9</td>
<td>4</td>
<td>340</td>
</tr>
<tr>
<td>[Fe2(OH)(O)L]4+</td>
<td>−1158.653119</td>
<td>9</td>
<td>4</td>
<td>592</td>
</tr>
</tbody>
</table>

[4] as two electron transfers occur in these systems. Partial charge redistribution can occur after peroxide → Fe interaction. Concomitant changes in the ligand iron distances lead to changes in the coordination of benzimidazole or pyridine with Fe. With DRS in the UV–VIS region, d–d, π–π* transitions, and charge transfer between metal and ligand such as metal-ligand charge transfer MLCT and ligand-metal charge transfer (LMCT) can be probed. The spectrum of [Fe2(HPTB)(OH)(NO3)2][NO3]2 shows λmax at 340 nm, due to the benzimidazole-iron charge transfer. The spectrum of [Fe2(HPTP)(OH)(NO3)2][ClO4]2 shows λmax at 290 nm, of a pyridyl-iron charge transfer. DRS-spectra of [Fe2(HPTB)(OH)(NO3)2][NO3]2 show their charge transfers at higher wavelengths compared to the [Fe2(HPTP)(OH)(NO3)2][NO3]2 complexes (Fig. 6). Maxima occur at 340 and 500 nm for [Fe2(HPTB)(OH)(NO3)2][NO3]2 and at 355 and 490 nm with a shoulder at 600 nm for the [Fe2(HPTP)(OH)(NO3)2][ClO4]2 complexes. From 300–400 nm oxo → Fe CT transitions are seen and for the bent Fe–O–Fe geometries three π-derived transitions of oxo pπ, pγ, pπ–Fe dz2, dxy and dx'y are expected. In the 440–510 nm, region pairs of absorption bands due to the bent Fe–O–Fe with 10-fold lower intensity are seen if carboxylato, carbonato or phosphate-bridges are present. Peroxo → Fe CT absorption arise between 400 and 500 nm upon addition of peroxides to the catalysts in methanol [26]. Both [Fe2(HPTP)(μ-OH)(NO3)2][ClO4]2 (1) and [Fe2(HPTB)(μ-OH)(NO3)2][ClO4]2 (2) form blue–violet adducts with H2O2. Easy formation of a 1:1 adduct of [Fe2(HPTB)(OH)(NO3)2][NO3]2 and
H₂O₂ are analogous to interaction of the reduced complex with O₂ in CH₂Cl₂ at −60°C to allow irreversible O₂ binding.

4. Conclusions

The reaction of the MMO mimic binuclear heptapodate coordinated iron(III)-complexes of N,N,N',N'-tetrakis(iminomethyl)-2-hydroxy-1,3-diamino-propane (HPTM) model with oxygen is studied. When one bridging ligand is removed from each ferrous iron the μ-η¹:η¹-O₂ binding proves the most favorable binding mode of dioxygen on complexes. This forms the so-called intermediate P (peroxo form), that can convert in the intermediate Q (bis-ferryl form) with little structural reorganization and this requires a moderate energy input of about 18.9 kcal/mol in order to facilitate the removal of two water molecules, the peroxo-bond cleavage and the formal oxidation of FeIII –Fe IV . The [Fe₂(HPTP)(μ-OH)]₄⁺ and [Fe₂(HPTB)(μ-OH)]₄⁺ model complexes are characterized with EXAFS to study the Fe–Fe and Fe-ligand bonding, the Fe–Fe distances are 3.020 and 3.223 Å, respectively, in accordance with the crystallographic data and the quantum mechanic value of 3.135 Å obtained on the [Fe₂(HPTM)(μ-OH)]₄⁺ model complexes. The σ- and π-bonds of the ferryl Fe=O in the plane of the Fe–O–Fe bridge, were analyzed and show that the nature of this ferryl species is this of a two atom three electron bond.

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References