Elucidation of mechanisms in manganese and iron based oxidation catalysis

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Chapter 2

A Computational Comparison of the Reactivity of non-Heme Fe(II) Complexes with NaOCl and H$_2$O$_2$.

Over the last decades, the involvement of Fe(III)-hypohalite, peroxido and hydroperoxido complexes in a myriad of essential enzyme catalysed halogenation and oxygenation reactions has been established and are central to the biosynthesis of natural products and antibiotics and in post translational modification of proteins. The dearth of spectroscopic data available on such hypohalite species, however, precludes their identification in biological systems and is in marked contrast to the body of data available on hydroperoxido species. Recently, our group reported the generation and spectroscopic characterization of nonheme Fe(III)-hypohalite intermediates of possible relevance to iron halogenases and demonstrated that Fe(III)-OCl polypyridyl amine complexes can be sufficiently stable at room temperature to be characterized by a UV/Vis absorption, resonance Raman and EPR spectroscopy and cryoESI-MS. In the present chapter, DFT methods are applied to rationalize the pathways to the formation of these Fe(III)-OCl, and ultimately Fe(IV)=O, species and provide indirect evidence for a short-lived Fe(II)-OCl intermediate. The species observed and the pathways involved are compared and contrasted with the related isoelectronic species generated from hydrogen peroxide.

2.1 Introduction

Halogenation of organic substrates, although relatively rare compared with oxygenation, is a central process in the biosynthesis of many natural products, and in the post translational modification of proteins;[1] typically these reactions are achieved by the activation of otherwise inert C-H bonds. Hence these reactions have attracted attention as they are of immediate interest and relevance to medicinal chemistry and biotechnology. Nature achieves halogenation by hijacking peroxidases and oxygenases to generate (vanadium and heme dependent) haloperoxidases and (non-heme Fe(II) dependent) halogenases, which use \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \), respectively.[2,3] The precise operation of this wide range of enzymes is of interest for biology and for the application of biomimetic complexes in C–H activation.

Nonheme iron halogenases have been proposed to react via radical pathways by transferring halide atoms (\( X^+ \)), whereas haloperoxidases use \( X^- \) in electrophilically.[1c,3b,4] For example, in the case of the heme haloperoxidases the reaction proceeds via formation of an Fe(IV)=O species, which reacts with a halide anion to form an Fe(III)–OX complex.[1c] The OX− formed can react directly or dissociates and is protonated to generate HOX near an enzyme bound substrate. In contrast, non-heme iron dependent halogenases are generally considered to generate Fe(IV)=O species, which engage in hydrogen abstraction from alkanes, with chlorination via the transfer of an iron bound chlorine atom equivalent.

Despite these mechanistic frameworks, major gaps remain in our understanding of their catalytic mode of action because of the rarity and the lack of spectroscopic data on species formed during such cycles. The transient nature and especially low steady state concentrations of the proposed reaction intermediates formed during enzyme catalyzed halogenation, such as Fe(II)–OCl, Fe(III)–OCl and, to a lesser extent, Fe(IV)=O, present a considerable challenge to their characterization and hence biomimetic model complexes are essential to the elucidation of the nature of these intermediates.[3] The intermediacy of related Fe(III)–O–IPh species is, however, precedent in the work of McKenzie and coworkers,[6a] and Nam and coworkers[6b,c] whereby the former study included the characterization of the intermediate by crystallography. A heme-Fe(III)-hypohalite was reported by Fujii,[7] Woggon[8] and coworkers, and Banse and coworkers made a tentative assignment of an intermediate as an Fe(II)-OCl species.[9] However, further than these data, the absence of spectroscopy on such species is in strong contrast to the wealth of data assembled regarding heme and nonheme high-valent Fe(IV)=O complexes.[10] This paucity of spectroscopic data limits our ability to identify such species under biologically relevant conditions and evaluate their kinetic competence in halogenations and hence the identification for the first time of the formation of a biomimetic nonheme iron(III)-hypohalite complex, i.e. \([\text{Fe(III)}(\text{OX})(\text{MeN4Py})]^{2+}\) (where MeN4Py = 1,1-di(pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)ethanamine and \( X = \text{Cl} \) or \( \text{Br} \) represented a breakthrough.[11] In our recent experimental study it was shown that iron(III)-hypohalite complexes form readily in water at low pH from \([\text{Fe(II)}(\text{OH}_2)(\text{MeN4Py})]^{2+}\) (Scheme 1)[12,13] when reacted with NaOCl or NaOBr at room temperature. The Fe(III)-OX species was found to undergo subsequent slower conversion to a relatively stable Fe(IV)=O analogue over several minutes.
non-heme Fe(II) complexes with NaOCl and H$_2$O$_2$

Scheme 1 Formation of [Fe(III)(OCl)(MeN4Py)]$^{2+}$ and [Fe(III)(OOH)(MeN4Py)]$^{2+}$ from [Fe(III)(OH$_2$)(MeN4Py)]$^{2+}$.

Before discussing computational characterization of this system it is useful to consider the experimental evidence for the formation and loss of the Fe(III)-OCl species. The UV/Vis absorption spectrum of [Fe(III)(OH$_2$)(MeN4Py)]$^{2+}$ exhibits visible absorption ($^1$MLCT) bands at ca. 387 and 490 nm (Figure 1a), between pH 1 and 6. Oxidation to [Fe(IV)(O)(MeN4Py)]$^{2+}$ with its characteristic NIR absorption band, NMR spectrum and resonantly enhanced Fe(IV)=O stretching band in its Raman spectrum is observed upon addition of either of the 2-electron oxidants H$_2$O$_2$ or HOCl in excess. These changes are consistent with observations made earlier by Banse and coworkers in the reaction of related Fe(II) complexes with NaOCl.

The visible absorption bands of [Fe(II)(OH$_2$)(MeN4Py)]$^{2+}$ disappeared upon addition of 0.5 equiv. of NaOCl (at pH 2.9) without a significant concomitant increase in absorption at longer wavelengths, e.g., of [Fe(IV)(O)(MeN4Py)]$^{2+}$ (Figure 1), and the formation of the one-electron oxidized complex Fe(III)-OH$^{[12b,c]}$ was apparent from the weak absorption band at ca. 480 nm and the characteristic EPR spectrum of a low spin Fe(III) complex (Figure 1a, inset), i.e. [Fe(III)(OH)(MeN4Py)]$^{2+}$. Addition of a further 0.5 equiv. of NaOCl (Figure 2a) resulted in an increase in absorbance at 480 nm ($\varepsilon = >500$ M$^{-1}$·cm$^{-1}$$^{[14]}$) followed by a decrease together with a concomitant increase in absorbance at 670 nm (Figure 2b). The latter absorbance is due to [Fe(IV)(O)(MeN4Py)]$^{2+}$.

The intermediate species was assigned as [Fe(III)(OCl)(RN4Py)]$^{2+}$, based on ESI-MS, its resonantly enhanced Fe-O and O-O and O-Cl stretching modes in the Raman spectrum at 473 nm at 580, 653 and 673 cm$^{-1}$ and the rhombic Fe(III) $S = \frac{1}{2}$ signal at 77 K (e.g., 2.17, 2.12 and 1.98 for [Fe(III)(OOH)(N4Py)]$^{2+}$$^{[13]}$.

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Figure 1 (a) UV/Vis absorption spectrum of \([\text{Fe(II)}(\text{OH}_2)(\text{MeN}_4\text{Py})]^{2+}\) (0.5 mM) at pH 2.2 at noted time intervals after addition of 0.5 equiv. of NaOCl at room temperature; inset EPR spectrum obtained from a flash frozen (to 77 K) sample taken at 300 s after addition of NaOCl. (b) Change in absorbance at 480 and 670 nm with log(time). Reproduced from ref [11] with permission. Copyright Wiley (2015).

Figure 2 (a) UV/Vis absorption spectrum of \([\text{Fe(II)}(\text{OH}_2)(\text{MeN}_4\text{Py})]^{2+}\) (0.5 mM) at pH 2.2 upon addition of a 2nd 0.5 equiv. of NaOCl at room temperature. (b) Change in absorbance at 480 and 670 nm with log(time). Reproduced from ref [11] with permission. Copyright Wiley (2015).

It was apparent from the time dependence of the changes in the UV/Vis absorption spectrum that \([\text{Fe(III)}(\text{OCl})(\text{MeN}_4\text{Py})]^{2+}\) does not form directly upon oxidation of \([\text{Fe(II)}(\text{OH}_2)(\text{MeN}_4\text{Py})]^{2+}\) but instead from a relatively slow ligand exchange between the Fe(III)-OH complex and NaOCl. [11,15] The formation of the Fe(III)-OCl species is followed by a slower reaction in which homolysis of the O-Cl bond occurs to yield the relatively stable Fe(IV)=O species. The formation and reactivity of this intermediate Fe(III)-OCl species was confirmed by a combination of cryo-ESI-MS, EPR and resonance Raman spectroscopy.

In this chapter a theoretical analysis and comparison of the reactions between \([\text{Fe(II)}(\text{OH}_2)(\text{MeN}_4\text{Py})]^{2+}\) and the isoelectronic 2-electron oxidations \(\text{H}_2\text{O}_2\) and HOCl is made. In particular the pathways followed to the formation of \([\text{Fe(III)}(\text{OX})(\text{MeN}_4\text{Py})]^{2+}\) species (where \(\text{OX} = \text{OCl or OOH}\) and the relative ease of homo- and hetero-lytic bond cleavage to form Fe(IV)=O species is explored. The goal of the present study is to determine the relative energies of the various possible spin states of the species proposed to be involved in these reactions and to use calculation of resonance enhancement of their Raman spectra to understand more deeply the electronic structure of the species. These data are essential as a basis for constructing reasonable models for the reactions that take place and in particular alternative pathways.
2.2 Results
Computational studies were performed to gain insight into the electronic features of the complexes and the reaction mechanisms involved in the formation and decomposition of the Fe(III)-OOH and Fe(III)-OCl species. Each structure described here was found through a full geometry optimization and characterized as a local minimum with a frequency calculation. To assist with the interpretation of experimental resonance Raman spectra (rR), we also calculated Raman spectra from the frequency calculations.

DFT calculations on species formed from 1 and 2 in water in Fe(II) and Fe(III) state
The calculation of energy and spin states was begun with an initial survey of functionals. Double hybrid density functionals were found to provide a good description of the equilibrium between HS and LS forms of the Fe(II)-OH$_2$ complexes, however, as a starting point the actual species present under reaction conditions need to be considered, i.e. Fe-OH$_2$ or Fe-OH. As the pH used in the HOCl reactions was below 4.5, the Fe(II)-OH$_2$ species was considered while the Fe(III)-OH (pKa = 1.9)$^{[128]}$ species is more relevant since the pH used was > 2.3. Calculation on the Fe(II)(CH$_3$CN) complex shows that it is much more stable than the aqua complexes, consistent with the observation that addition of even 1% by volume of CH$_3$CN is sufficient to return the complex to the Fe(II)(CH$_3$CN) state. In this section the energies of various spin states of the species of relevance to the reactions of 1 and 2 with NaOCl and with H$_2$O$_2$ are discussed and in particular the prediction of resonance enhancement, as it is a key tool in speciation studies.

DFT calculations on [Fe(IV)(MeN$_4$Py)=O]^{2+}
The [Fe(IV)(MeN$_4$Py)=O]^{2+} species was prepared and isolated through chemical oxidation of [Fe(II)(MeN$_4$Py)OH$_2$]^{2+} in water under acidic conditions.$^{[16]}$ The UV/Vis absorption spectrum shows a characteristic band at 680 nm and its Raman spectrum at $\lambda_{\text{exc}}$ 785 nm and at $\lambda_{\text{exc}}$ 473 nm show resonant enhancement of the [Fe(IV)(MeN$_4$Py)=O]^{2+} stretching mode with the expected $^{16/18}$O isotope shift from 843 to 807 cm$^{-1}$; i.e. a 36 cm$^{-1}$ shift, which is in good agreement with the calculated shift (37 cm$^{-1}$), using a two atom approximation. The triplet spin state of [Fe(IV)(O)(MeN$_4$Py)]$^{2+}$ was calculated to be 8.7 kcal/mol more stable than the quintet, while the singlet state lies at far higher energy (31.4 kcal/mol above the triplet state). Hence, for thermodynamic reasons calculation of rRaman spectra for higher spin states was not attempted.

![Figure 3 Left: DFT-calculated rR spectrum of the Fe(IV)=O (triplet) species (excited at 911 nm). The mode that shows the greatest degree of enhancement is at 884 cm$^{-1}$ with a mode enhanced to a lesser extent at 845 cm$^{-1}$ (inset: calculated absorption spectrum). The former mode involves primarily an Fe-O stretching displacement. Right: structure and relative spin states free energies.](image-url)
DFT calculations on [Fe(III)(MeN4Py)-OOH]$^{2+}$

The characterization Fe(III)-OOH species was already reported for RN4Py complex.\textsuperscript{[17]} Roelfes et al. assigned for the [Fe(III)(N4Py)-OOH]$^{2+}$, by rR ($\lambda_{\text{exc}}$ 647.1 nm), a O-O stretching vibration at 790 cm$^{-1}$ and a Fe-O vibrational mode at 632 cm$^{-1}$. The calculated results instead propose a O-O stretching found at 753 cm$^{-1}$ and a backbone Fe-N vibration at 861 cm$^{-1}$ but the Fe-O stretching appears to be only at 608 cm$^{-1}$ and seems to be enhanced at $\lambda_{\text{exc}}$ 572 nm only (Figure 6). The vibrational modes are underestimated due to the harmonic approximation and the fact that calculations were carried out in the gas phase.

Figure 4 (left) Resonance Raman spectra at various excitation wavelengths of 1 (1 mM) in methanol after addition of 50 equiv. H$_2$O$_2$. (right) the corresponding UV/vis absorption spectrum.

Figure 5 Left: DFT-calculated Resonance Raman spectra of the sextet (red spectrum) and doublet (blue spectrum) spin states of [Fe(III)(MeN4Py)-OOH]$^{2+}$ calculated respectively at 312 nm and 355 nm excitation. Right: structure of the [Fe(III)(MeN4Py)-OOH]$^{2+}$ species and the relative spin states free energies.
The ground spin state of this species is the doublet (Figure 4), however the difference in the free energies (under normal conditions) between the sextet and doublet is only 1.3 kcal/mol in favour of the doublet. The small difference between the high and low spin energies required that both are calculated since the resonance Raman spectrum is likely a representation of either a combination or only one of the two molecular electronic configurations.

DFT calculations of [Fe(III)(MeN4Py)-OH]$^{2+}$
Calculation of the energies of the various states of the [Fe(III)(MeN4Py)-OH]$^{2+}$ species is limited by the difficulty in observing the species by resonance Raman spectroscopy. The reason for the lack of enhancement is probably due to the equilibrium between low and high spin states. The calculated free energy difference under normal conditions (SATP) between doublet and sextet is 1.34 kcal/mol. The difference between those two spin states is probably small enough that minor small environmental changes (temperature or pH) are enough to switch the relative abundance of the doublet in solution, which seems to give a stronger enhancement of the Fe-ligands modes. Amongst the enhancement bands, calculated for excitation at 330 nm for the doublet spin state are the a O-H bending mode (859 cm$^{-1}$), an Fe-O stretching mode at (640 cm$^{-1}$), and Fe-N(amino) stretching mode (577 cm$^{-1}$).
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DFT calculations of [Fe(III)(MeN4Py)-OCl]^{2+}
The intermediacy of the [Fe(III)(MeN4Py)-OCl]^{2+} species means that only resonance Raman data can be obtained for this complex (i.e. since it cannot be isolated in concentrated or solid form then a non-resonant Raman or IR spectrum is not available). A shift of the bands of [Fe(III)(OCl)(MeN4Py)]^{2+} from 653 and 580 cm^{-1} to 628 and 562 cm^{-1}, respectively, was observed experimentally upon $^{18}$O labelling. The observed shift of 25 and 18 cm^{-1}, respectively, are close to those expected for an Fe-O bond (29 cm^{-1}) and O-Cl (23 cm^{-1}) modes. The former band is characteristic for an Fe-O stretching mode and the latter that of an O-Cl stretching mode. Notably, the band at 673 cm^{-1} is unperturbed by the use of Na$^{18}$OCl but undergoes a shift (from 673 to 676 cm^{-1}) when NaOBr was used$^{[18]}$ (Figure 9), which is consistent with the assignment of the band as a symmetric Fe-O-Cl bending mode.

Figure 8 Resonance Raman DFT simulation of [Fe(III)(MeN4Py)-OH]^{2+}. The sextet (blue line) and the doublet (red line) were both simulated around the 355 nm region.
non-heme Fe(II) complexes with NaOCl and H₂O₂

Figure 9 Resonance Raman spectra at λ_{exc} 473 nm of intermediates generated upon the reaction of [(MeN₄Py)Fe(II)(Cl)]Cl (4 mM at pH 2.2) with (a) Na^{16}OCl in ^{16}OH₂ and (b) Na^{18}OCl in ^{18}OH₂. Reproduced from ref [11] with permission. Copyright Wiley (2015).

Figure 10 Resonance Raman spectra of [(MeN₄Py)Fe(II)(Cl)]Cl (4 mM in H₂O at pH 2.2) with (a) NaOCl and (b) NaOBr at λ_{exc} 473 nm. Reproduced from ref [11] with permission. Copyright Wiley (2015).

Figure 11 Simulation of the rR spectrum of the Fe(III)-OCl species (excitation at 502 nm). The modes that show the greatest degree of enhancement are: 549 cm⁻¹ (O-Cl stretching mode), 572 cm⁻¹ (N_{amino}-Fe stretching mode), 592 cm⁻¹ (Fe-O-Cl Bending mode), 644 cm⁻¹ (symmetric Py-Fe breathing), 658 cm⁻¹ (Py ring breathing with minor influence of the OCl moiety), 853 cm⁻¹ (N_{amino} bending mode).
The resonance Raman spectrum of \( [\text{Fe(III)}(\text{OCl})(\text{MeN4Py})]^2+ \) was calculated by DFT methods (Figure 11). The calculated spectrum shows that although these modes involve a larger part of the complex than the Fe-O-Cl core, the assignment of bands to Fe-O-Cl stretching and bending vibrations is, to a first approximation, reasonable.

In summary, the calculated resonance Raman spectra give only a qualitative description of the system but nevertheless support that the validity of the calculation of the spin state ordering, which is a prerequisite for correct description of reaction mechanisms.

**Reaction of \( [\text{Fe(II)}(\text{OH}_2)(\text{MeN4Py})]^2+ \) with \( \text{H}_2\text{O}_2 \) and \( \text{HOCl} \)**

The formation of the \( [\text{Fe(III)}(\text{OOH})(\text{MeN4Py})]^2+ \) and \( [\text{Fe(III)}(\text{OCl})(\text{MeN4Py})]^2+ \), and thereafter \( [\text{Fe(IV)}(\text{O})(\text{MeN4Py})]^2+ \), upon reaction of \( [\text{Fe(II)}(\text{OH}_2)(\text{MeN4Py})]^2+ \) with \( \text{H}_2\text{O}_2 \) and with \( \text{NaOCl} \), respectively, in water [11] was confirmed spectroscopically and is proposed to occur in organic solvents also. [17a] DFT calculations were carried out using models and methods benchmarked and calibrated earlier [19] to provide insight into free energy changes (relative to \( [\text{Fe(II)}(\text{OH}_2)(\text{MeN4Py})]^2+ \), labelled \(^5\text{A}\) in the schemes below for each of the proposed steps in the mechanism that leads to the formation of the Fe(III)-OOH (\(^6\text{D}\)) or Fe(III)-OCl (\(^6\text{D}^*\)) and, ultimately, the Fe(IV)=O (\(^3\text{E}\)) complexes.

**Ligand exchange and initial formation of \( [\text{Fe(IV)}(\text{O})(\text{MeN4Py})]^2+ \)**

The overall mechanism by which these species form is summarised in Scheme 2. The initial step, displacement of water by \( \text{H}_2\text{O}_2 \) and by \( \text{NaOCl} \) is rapid, consistent with the large driving force calculated for each process. However, the formation of a Fe(IV)=O species has not been observed experimentally, which is understandable considering the extremely large driving force for this latter heterolysis step. Indeed, although the activation barriers to these latter reactions has not been calculated, the reactions are highly exergonic (ca. 30 kcal mol\(^{-1}\)), driven largely by the formation of the energetically stable species (OH\(^-\) and Cl\(^-\)).
non-heme Fe(II) complexes with NaOCl and H$_2$O$_2$

Scheme 2 Reaction scheme showing the formation and decomposition of [Fe(II)(OOH)(MeN4Py)]$^{2+}$ and [Fe(II)(OCl)(MeN4Py)]$^{2+}$ to [Fe(IV)(O)(MeN4Py)]$^{2+}$ and calculated free energies for each step. Data includes dispersion, entropic and solvent corrections and is given in kcal mol$^{-1}$.

Comproportionation of [Fe(IV)(O)(MeN4Py)]$^{2+}$ with [Fe(II)(OH$_2$)(MeN4Py)]$^{2+}$

The [Fe(IV)(O)(MeN4Py)]$^{2+}$ formed will, in each case, comproportionate rapidly with [Fe(II)(OH$_2$)(MeN4Py)]$^{2+}$, a reaction which is most likely diffusion limited (Figure 11) and is also calculated to have a large driving force.

Figure 11 Addition of an aqueous solution of [[MeN4Py]Fe(IV)(O)](PF$_6$)$_2$ (2 mM, 10 µL per addition) to an aqueous solution of [[MeN4Py]Fe(II)(CH$_3$CN)](ClO$_4$)$_2$ (0.25 mM, 1 mL, with stirring) in water (inset: addition of 1 equiv of [[MeN4Py]Fe(IV)(O)](PF$_6$)$_2$ results in complete disappearance of the absorption bands of the iron(II) complex, further addition of [[MeN4Py]Fe(IV)(O)](PF$_6$)$_2$ leads to an increase in its characteristic absorption at 670 nm). Spectra are corrected for dilution.
Ligand exchange and initial formation of [Fe(III)(OH)(MeN4Py)]²⁺
The reaction between the Fe(III)-OH complex (⁶C) formed and H₂O₂ was calculated to be mildly exergonic (7.0 kcal mol⁻¹), while in contrast the reaction with NaOCl is endergonic (7.0 kcal mol⁻¹). Nevertheless, in both cases the build-up of the Fe(III)-OX species (X = -OH or -Cl) is facilitated by the subsequent highly endergonic homolytic cleavage step (15 and 20.7 kcal mol⁻¹, respectively) that converts the Fe(III)-OOH (⁶D) and Fe(III)-OCl (⁶D*) to the Fe(IV)=O species (⁶E). Indeed both species can be observed transiently in solution. The absence of detectable amounts of Fe(III)-OCl above pH 5 (i.e. where the concentration of OH⁻ becomes significant) is in agreement with the conclusion that Fe(III)-OH (⁶C) is in equilibrium with Fe(III)-OOH (⁶D) and Fe(III)-OCl (⁶D*) are in equilibrium with each other. Indeed, reaction of the Fe(III)-OH complex (⁶C), prepared independently, with H₂O₂ or NaOCl shows that the Fe(III)-OX species form rapidly to reach an equilibrium with Fe(III)-OH more rapidly than they convert to [Fe(IV)(O)(MeN4Py)]²⁺.¹¹

The overall reactions are summarized in Schemes 5 and 6 below.

Scheme 3 Comproportionation between [Fe(II)(OH)(MeN4Py)]²⁺ and [Fe(IV)(O)(MeN4Py)]²⁺ and calculated free energies for the reaction. Data includes dispersion, entropic and solvent corrections and is given in kcal mol⁻¹.
non-heme Fe(II) complexes with NaOCl and H₂O₂

Scheme 5 Reaction scheme showing the formation of and relation between [Fe(III)(OOH)(MeN4Py)]²⁺ and [Fe(IV)(O)(MeN4Py)]²⁺ and calculated free energies for each step. Data includes dispersion, entropic and solvent corrections and is given in kcal mol⁻¹.
Scheme 6 Reaction scheme showing the formation of and relation between $[\text{Fe(III)}(\text{OCl})(\text{MeN}4\text{Py})]\text{2+}$ and $[\text{Fe(IV)}(\text{O})(\text{MeN}4\text{Py})]\text{2+}$ and calculated free energies for each step. Data includes dispersion, entropic and solvent corrections and is given in kcal mol$^{-1}$.

2.3 Conclusions

In summary, for the first time a non-heme iron(III)-hypochlorite complex was identified in acidic aqueous solution at room temperature. Importantly, it is found that a non-heme Fe(IV)=O species forms from both an Fe(III)-OOH and an Fe(III)-OCl complex by homolytic cleavage of the O-O and O-Cl bonds. Furthermore, the formation of Fe(II)-OOH and Fe(II)-OCl species initially is energetically plausible given the exergonicity of the reactions. This reactivity is notable since the reverse reaction, i.e. the formation of hypohalites by reaction of Fe(IV)=O species with halides, is generally viewed as a key step in the halogenation of organic substrates.$^{[1]}$ In the case of iron halogenases the formation of intermediate Cl-Fe(IV)=O species have been proposed in which the Fe(IV)=O moiety abstracts a hydrogen atom followed by attack of the carbon radical formed on the bound chlorido ligand. Hence, the observation of a non-heme Fe(III)-OCl species and of a pathway to a potentially C–H abstracting Fe(IV)=O that involves concomitant formation of an hydroxyl or chlorine radical, holds implications in regard to our understanding of the mechanisms by which iron haloperoxidases and halogenases operate.

Furthermore, although Fe(III)-OCl species can be observed, it is clear that such species are at best transient and may be regarded as spectroscopically analogous to the corresponding Fe(III)-
non-heme Fe(II) complexes with NaOCl and H₂O₂

OH and Fe(III)-OOH complexes. The key differences identified here in the UV/Vis absorption, Raman and EPR spectra provides an important basis of spectral characteristics with which to identify such species if present as actors in the biosynthesis of halogenated natural products.

Overall the mechanisms for reaction of 1 with H₂O₂ and with NaOCl are essentially the same. In each case the initial reaction of the two electron oxidation with 1 is heterolytic cleavage to generate the Fe(IV)=O species. Rapid comproportionation leads to quantitative conversion to the corresponding Fe(III)-OH species. This latter species then coordinates to the oxidants reversibly and undergoes homolytic OO and O-Cl bond cleavage to form Fe(IV)=O species and hydroxyl and chlorine radicals. The relative stability of the Fe(IV)=O species indicates that the reactive species, in terms of substrate oxidation, in each case is the radical formed that opens the path to a rebound mechanism with the Fe(IV)=O species. In the next chapter the reactivity of 1 with oxygen and the formation of similar species will be discussed.

2.4 Experimental Section

Resonance Raman spectra were calculated for the Fe(III)-OCl (doublet) and the Fe(IV)=O (triplet) species using ORCA (version 3.0.2) and the IMDHO method as implemented in the orca_asa program. The procedure used was as follows: The geometry optimization and the Hessian matrix (frequency) were calculated with the Becke88[22] and Perdew86[23] (BP86) DFT functional using Def2-TZVP basis set. The Hessian matrix, generated by the frequency calculations, was used in combination with the TD-DFT[25] calculations, which were performed using the BH and HLYP functional with the TZVPP[24] basis set for Iron and SV(P)[24]a basis set for C, H, N, O and Cl, to calculate the rRaman enhancements for each normal mode.

All the calculations for energy landscapes and ¹⁸O isotope shifts were performed using Density Functional Theory (DFT) with the unrestricted B3LYP method[27] as implemented in the Gaussian 09c program package. Single point calculations with B3LYP-D3[28] were calculated using Jaguar V3.0 Rev 2. All calculations utilized a triple-ζ quality basis set that includes LANL2TZ+(f) on iron and 6-311+G(d,p) on the rest of the atoms. Free energies (ΔG) reported here use UB3LYP-D3 energies corrected with ZPE, thermal and entropic corrections from the frequency calculation at 298 K. The effect of solvent was tested through single point calculations using water as a solvent with the SMD solvation model as implemented in Gaussian.

Further information for the calculated molecules (i.e. coordinates) can be obtained in the supporting information of the article


2.5 References


[13] The Fe(II) complexes [Fe(II)(CH2CN)(N4Py)]2+ and [Fe(II)(Cl)(MeN4Py)]2+ undergo immediate solvolysis in water to form the corresponding [Fe(II)(OH)2(N4Py)]2+ and [Fe(II)(OH)2(MeN4Py)]2+ complexes at pH ca. 3, respectively.[12b]

[14] The value is an estimate based on the assumption of a maximum of 40% conversion of the Fe(III) present being in the form of Fe(III)-OCl. The value is consistent with the molar absorptivity of related Fe(III)-O(OH) complexes. R. Y. N. Ho, L. Que Jr., G. Roelfs, B. L. Feringa, R. Hermant, R. Hage, *Chem. Commun.* **1999**, 2161.

[15] Essentially, the same trend in the time dependence of spectroscopic properties were obtained with the related complex [Fe(II)(OH2)2(N4Py)]2+ (where N4Py = 1,1-dil(pyridin-2-yl)-N,N-bis(pyridin-2-


[18] NaOBr was prepared from aqueous NaOCl and NaBr (Figure S14 of ref [11]).


