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Resonance Raman evidence for the interconversion between an $[Fe^{III}–\eta^1\text{-}OOH]^{2+}$ and $[Fe^{III}–\eta^2\text{-}O_2]^+$ species and mechanistic implications thereof

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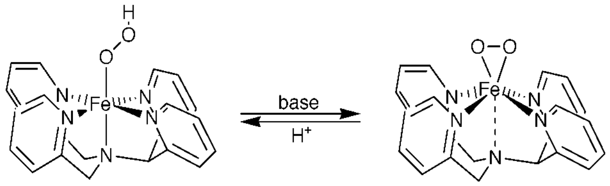
The deprotonation of $[Fe^{III}(N4Py)(\eta^1\text{-}OOH)]^{2+}$ 1 gives $[Fe^{III}(N4Py)(\eta^2\text{-}OO)]^+$ 2, as unequivocally demonstrated by resonance Raman spectroscopy, and leads to the loss of alkane hydroxylation activity by 1.

Iron–peroxo species have been established or postulated in the chemistry of dioxygen activation at mononuclear iron centres in biology.[1,2] For example, activated BLM,† the form of the antitumor drug bleomycin responsible for its DNA cleavage activity,[3,4] is formulated as a low spin $Fe^{III}–\eta^1$-OOH species.[5] Similarly, an $Fe^{III}–\eta^1$-OOH moiety is strongly implicated in the mechanism of cytochrome P450 and considered the key intermediate immediately prior to the cleavage of the O–O bond to generate the high valent iron–oxo oxidant.[6] Both $Fe^{III}–\eta^1$-OOH species are proposed to derive from the one-electron reduction of oxy adducts ($[Fe–O_2]^{2+}$) and subsequent protonation, possibly *via* an $[Fe^{III}–\eta^2\text{-}O_2]^+$ species. Such $[Fe^{III}–\eta^2\text{-}O_2]^+$ species are also postulated to be the source of nucleophilic peroxides that are implicated in substrate oxidations by heme enzymes such as aromatase[7] and heme oxygenase.[8] A related $[Fe^{III}–\eta^2\text{-}O_2]^+$ species has been suggested to account for the *cis*-dihydroxylation chemistry of the Rieske dioxygenases.[9] Significant effort has been devoted in the characterisation of small molecule analogues of such peroxo intermediates. Two high spin $[Fe^{III}–\eta^2\text{-}O_2]^+$ species, namely $[Fe^{III}(EDTA)(O_2{}^{2-})]$[10,11] and $[Fe(porphyrin)O_2]^-$,[12] have been identified, while the first examples of synthetic $Fe^{III}–\eta^1$-OOH species have just appeared in the last few years.[13–15] We have sought to establish a precedent for the interconversion between an $[Fe^{III}OOH]^{2+}$ species and its deprotonated form $[Fe^{III}O_2]^+$, implicit in the mechanisms of bleomycin and cytochrome P450. Such an equilibrium was recently reported for Fe^{III}(N-R-trispicen) complexes (R = Me, Et, Bn),[16] but no Raman data was provided to establish the binding mode of the $[Fe^{III}O_2]^+$ species. Here, we provide unequivocal Raman evidence for the η^2-peroxo binding in the $[Fe^{III}(N4Py)O_2]^+$ species, obtained from the deprotonation of the $[Fe^{III}(N4Py)(\eta^1\text{-}OOH)]^{2+}$ species. In addition we show for the first time that protonation of the $[Fe^{III}O_2]^+$ species is essential for alkane oxidation.

The purple $[Fe^{III}(N4Py)(OOH)]^{2+}$ complex **1** (λ_{max} 547 nm, ε_M 1300 dm^3 mol^{-1} cm^{-1}) is generated at −20 °C by addition of 5 equiv. H_2O_2 to a methanolic solution of $[Fe^{III}(N\text{-}4Py)(OMe)]^{2+}$, obtained by pretreating $[Fe^{II}(N4Py)(MeCN)]^{2+}$ with 5 equiv. H_2O_2 at room temperature.[15] Upon addition of 5 equiv. NH_3 (aq) at −45 °C, the purple chromophore converts to a blue species **2** (Scheme 1) with an absorption maximum at 685 nm (ε_M 520 dm^3 mol^{-1} cm^{-1}). Addition of perchloric acid reverses this colour change and quantitatively regenerates the original purple solution. The conversion of **1** to **2** can also be monitored by EPR spectroscopy. Addition of base converts the characteristic low-spin Fe^{III} EPR spectrum of **1** with *g* values at 2.3, 2.1 and 1.9 to one with an intense isotropic signal at g = 4.3, which is typical of a mononuclear rhombic high-spin Fe^{III} center (E/D = 1/3). In the case of the Fe^{III}(N-R-trispicen) complexes, the addition of base to the Fe^{III}–OOH species converts them to high-spin Fe^{III} complexes with more axial EPR spectra ($E/D \approx 0.08$).[16]



1 2

Scheme 1 Proposed structures for **1** and **2**.

Electrospray ionisation mass spectrometry provides the elemental composition of **2**. As reported earlier, the ESMS of **1** shows a prominent feature at *m/z* 555, whose mass and isotope distribution pattern unequivocally assigns this ion as $\{[Fe(N\text{-}4Py)(OOH)]ClO_4\}^+$.[13a] The ESMS of **2**, on the other hand, exhibits a prominent feature at *m/z* 455, a value which corresponds to the loss of $HClO_4$, relative to that of **1**. The isotope distribution pattern associated with this feature matches well with that calculated for, $[Fe(N4Py)(OO)]^+$, thus supporting the notion that **2** is the conjugate base of **1**.

Resonance Raman spectroscopy provides further insight into this transformation. Excitation into the peroxo-to-iron(III) charge transfer band of **1** generates a Raman spectrum with features at 632 and 790 cm^{-1} [Fig. 1(*a*)].‡ The latter is unequivocally assigned to ν(O–O) by its ^{18}O shift of −46 cm^{-1}, while the former is associated with a coupled Fe–OOH mode.[14] These features are considered the Raman signature for a low spin Fe(III)–OOH species. Conversion to **2** results in a significantly different Raman spectrum with prominent peaks at 495 and 827 cm^{-1} [Fig. 1(b)].§ The use of $H_2{}^{18}O_2$ shifts the two features to 478 ($\Delta\nu$ = −17) and 781 ($\Delta\nu$ = −46) cm^{-1}, respectively [Fig. 1(c)], which are fully consistent with their assignments as ν(Fe–O$_2$) ($\Delta\nu_{calc}$ −18 cm^{-1}, based on Hooke's Law for diatomic harmonic oscillators) and ν(O–O) ($\Delta\nu_{calc}$ −47 cm^{-1}), respectively. Consistent with these assignments, the introduction of ^{54}Fe in **2** does not affect the 827 cm^{-1} vibration but upshifts the 495 cm^{-1} feature by 3 cm^{-1} ($\Delta\nu_{calc}$ +3 cm^{-1}) [Fig. 1(d)]. Furthermore, unlike for **1**,[14] the use of $^2H_2O_2$ does not affect the Raman spectrum of **2**. Thus the Raman spectra support the notion that **2** is an $[Fe^{III}–O_2]^+$ complex.

Strong evidence for the η^2-peroxo binding mode has been obtained from the Raman spectrum of **2** with H_2O_2 containing 61% ^{18}O (Fig. 2).¶ Although the various isotopic components of

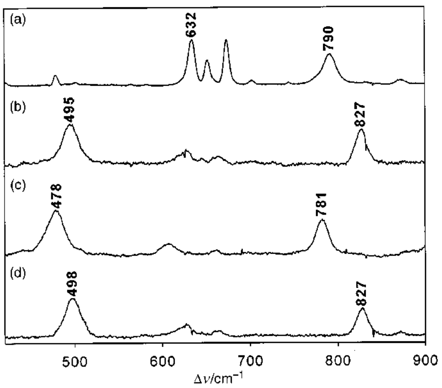


Fig. 1 Resonance Raman spectra of **1** and **2**. (a) **1** generated by the addition of 5 equiv. H_2O_2 to a methanolic solution of $[Fe^{III}(N4Py)(OMe)]^{2+}$ (10 mM) at −35 °C. (b) **2** generated by the addition of 5 equiv. NH_3(aq) to the solution of **1** at −35 °C. (c) Same as (b), except $H_2{}^{18}O_2$ was used. (d) Same as (b) except that the ^{54}Fe complex was used. All spectra were obtained with a backscattering geometry on liquid-N_2 frozen samples using 568.2 nm laser excitation at 20 mW power at the sample. The Raman frequencies were referenced to indene.

the ν(Fe-O_2) feature centred at 486 cm^{-1} are unresolved, three peaks associated with the ν(O–O) feature are readily discerned at 781, 802 and 826 cm^{-1} and can be fitted with peaks having approximately equal linewidths. The fact that the peak arising from the $^{16}O^{18}O$ isotopomer has a linewidth equal to those of $^{16}O^{16}O$ and $^{18}O^{18}O$ isotopomers strongly implies an η^2-peroxo binding mode, as found for $[Fe^{III}(EDTA)(\eta^2\text{-}O_2)]$[10,11] and suggested for $[Fe^{III}(N\text{-}R\text{-trispicen})(O_2)]^+$.[16] At this point, we do not have enough information to determine whether the iron centre in **2** becomes seven-coordinate, as illustrated in Scheme 1, or remains six-co-ordinate by a decrease in the denticity of the N4Py ligand. The latter may come about by detachment of one of the pendant pyridines or, by breaking the already weak tertiary amine bond as the η^2-peroxo ligand pulls the iron centre further out of the plane defined by the four pyridine ligands to achieve a coordination geometry similar to that found in the structure of $[Mn(TPP)(\eta^2\text{-}O_2)]^-$.[17]

With the peroxo binding mode of **2** established, we now compare the relative abilities of **1** and **2** to oxidise substrates. We have previously found that $[Fe^{II}(N4Py)(MeCN)]^{2+}$ can catalyse the hydroxylation of cyclohexane with H_2O_2 in acetone *via* intermediate **1**.[13a] With 5 equiv. H_2O_2, we observe 1.6 and 0.4 turnovers of cyclohexanol and cyclohexanone, respectively. In CD_3OD the number of turnovers is lower, 0.8 and 0.4 turnovers, respectively, owing to a competing oxidation of the solvent to $D_2C{=}O$ (1.4 turnovers). When 5 equiv. NH_3 (aq) is added prior to or after the formation of **1** in CD_3OD, no oxidation of cyclohexane is observed. These results suggest that, whereas **1** is capable of activating the O–O bond to oxidise alkanes, **2** is unreactive towards such substrates, resembling the inertness of other $[Fe^{III}(\eta^2\text{-}O_2)]$ species.[11,18] Our demonstration of an $[Fe^{III}(\eta^1\text{-}OOH)]/[Fe^{III}(\eta^2\text{-}OO^-)]$ interconversion allows us to support unequivocally the hypothesis that protonation of the peroxo species activates it for participation in the oxygen activation mechanisms by iron enzymes.[6,11]

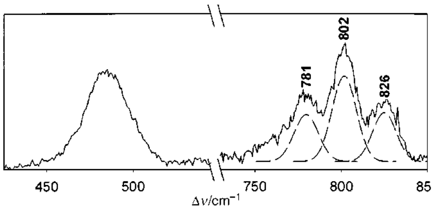


Fig. 2 Raman spectrum of **2** generated with a statistical mixture of H_2O_2 isotopomers (61% ^{18}O). The dashed lines represent the curve fit of the features associated with the ν(O–O) peaks. The curve was fitted with three peaks with the $^{18}O^{18}O$ feature constrained to a frequency of 781 cm^{-1} and a linewidth of 16 cm^{-1} as found for $[Fe(N4Py)(^{18}O_2)]^+$. The fitted data gave the other two peaks at 802 and 826 cm^{-1} with linewidths of 16.3 and 15.9 cm^{-1}, respectively. The latter matched well the properties of the ν(O–O) feature in $[Fe(N4Py)(^{16}O_2)]^+$.

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Notes and references

† Abbreviations used: BLM = bleomycin; N4Py = *N*-[bis(2-pyridyl)methyl]-*N,N*-bis(2-pyridylmethyl)amine; N-R-trispicen = *N*-alkyl-*N, N′,N′*-tris(2-pyridylmethyl)ethane-1,2-diamine.

‡ Resonance Raman spectra were collected on an Acton AM-506 spectrometer (2400-groove grating) using Kaiser Optical holographic super-notch filters with a Princeton Instruments liquid N_2-cooled (LN-1100PB) CCD detector with 4 or 2 cm^{-1} spectral resolution. Spectra were obtained by back-scattering geometry on liquid N_2 frozen samples using 568.2 nm laser excitation from a Spectra Physics 2030-15 argon ion laser and a 375B CW dye (Rhodamine 6G). Raman frequencies were referenced to indene.

§ Aside from the features at 495 and 827 cm^{-1}, weaker features at 625 , 645, 664 cm^{-1} are also observed and are sensitive to ^{18}O labeling. However the intensities of these features vary relative to the intensities of the 495 and 827 cm^{-1} features with different samples, suggesting that these minor features are not associated with **2**. We have ruled out the possibility that these minor features are associated with **1** since the features at 626, 648, and 668 cm^{-1} are slightly shifted from those of **1** and a corresponding 790 cm^{-1} feature is not observed. The assignment of these features is currently under investigation.

¶ $H_2{}^{18}O_2$ (90% ^{18}O-enriched, 2% solution in $H_2{}^{16}O$) was obtained from ICON Services Inc. The statistical mixture of H_2O_2 (61% ^{18}O) was prepared by the reduction of O_2 (statistical mixture with 61% ^{18}O from ICON Services Inc.) according to a literature procedure (A. J. Sitter and J. Terner, *J. Labelled Compd. Radiopharm.*, 1984, **22**, 461).

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