LETTER

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Intramolecular aromatic hydroxylation mediated by a dinuclear iron complex: an oxo-Fe^{IV} Fe^{IV} active intermediate is suggested†

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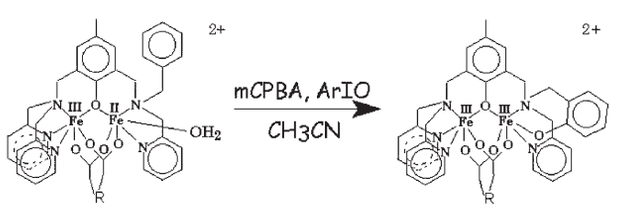
In the presence of oxygen atom donors [XO, *e.g.*, *m*-chloroperbenzoic acid (*m*-CPBA), *o*-*tert*-butylsulfone iodosyl benzene (ArIO)] the benzyl group of the ligand in a mixed-valent Fe^{II}Fe^{III} complex is almost quantitatively *ortho*-hydroxylated to a phenolate terminally bound to one iron in the derived Fe^{III}Fe^{III} complex. All available experimental evidence concurs to suggest that this reaction involves an oxo-Fe^{IV}Fe^{IV} intermediate.

Non-heme iron oxygenases have received much attention in the recent past owing to their ability to catalyze a wide range of oxygen transfer and oxidation reactions, in particular aliphatic and aromatic hydroxylations.[1–3] Examples of non-heme iron aromatic hydroxylases include enzymes with both mononuclear (*e.g.*, phenylalanine hydroxylase[4]) and dinuclear (*e.g.*, toluene-2-monooxygenase[5]) active sites. Model systems have been developed in parallel to contribute to an understanding of their mechanisms of action and eventually to mimic their activities. In particular, a few model complexes have been reported to perform aromatic hydroxylations when treated with dioxygen under reducing conditions or with an activated oxygen surrogate such as hydrogen peroxide, an alkylhydroperoxide or an iodosyl arene.[6–8] Most of them are or involve mononuclear species. A single well-characterized dinuclear system active in oxygen transfer was reported in the literature but detailed mechanistic information has not been provided yet.[8]

In the course of our studies of dinuclear iron systems, we reported the characterization of [Fe^{II}Fe^{III}(L)(mpdp)(H$_2$O)]·(ClO$_4$)$_2$ (mpdp = *m*-phenyldipropionate), a mixed-valent Fe^{II}-Fe^{III} complex of a hexadentate phenolato ligand (HL) bearing a dangling benzyl group (Scheme 1), which leaves a solvent accessible coordination site on the iron pair.[9,10] In this letter, we report that in the presence of oxygen atom donors [XO, *e.g.*, *m*-chloroperbenzoic acid (*m*-CPBA), *o*-*tert*-butylsulfone iodosyl benzene (ArIO)[11]] the benzyl group of the ligand is almost quantitatively *ortho*-hydroxylated to a phenolate terminally bound to one iron in [Fe$_2$(L − H + O)(mpdp)]$^{2+}$. All available experimental evidence concurs to suggest that this reaction involves an oxo-Fe^{IV}Fe^{IV} intermediate.



Scheme 1 For mpdp R = 1,3-xylyl

When a blue acetonitrile solution of [Fe^{II}Fe^{III}(L)(mpdp)(CH$_3$CN)]$^{2+}$ was treated with 4 equiv. of *m*-CPBA, it immediately turned deep turquoise blue, in agreement with both an intensity increase and a shift of the maximum wavelength from 580 to 610 nm.

This transformation was complete within 2 min at room temperature. Electrospray ionization mass spectrometry (ESI-MS) analysis showed (Fig. S1 in the Electronic supplementary information) that the starting compound exhibited peaks at *m*/*z* 430 {[Fe^{II}Fe^{III}(L)(mpdp)]$^{2+}$} and 959 {[Fe^{II}Fe^{III}-(L)(mpdp)(ClO$_4$)]$^{+}$}. During the reaction these peaks were replaced by new ones (see spectrum b in Fig. 1) at *m*/*z* 437.5, 875, 974 and two other peaks at 384.5 and 769. The latter peaks are associated with the N-debenzylation of the ligand, as already observed in some cases.[12] The three other peaks show that the main product of the reaction has a mass increase of 15, which corresponds to the fixation of an oxygen atom combined with the loss of a proton ([Fe$_2$(L − H + O)(mpdp)]). The three peaks are assigned to the respective formulas [Fe^{III}Fe^{III}(L − H + O)(mpdp)]$^{2+}$, [Fe^{II}Fe^{III}(L − H + O)(mpdp)]$^{+}$ and [Fe^{III}Fe^{III} (L − H + O)(mpdp)·(ClO$_4$)]$^{+}$.

Multiple-stage tandem ESI-MS analysis of the ligand recovered after extraction of the iron (Fig. S2 in the ESI) indicated that the oxygenation occurred on the benzyl group. Indeed, by fragmentation of the isolated ligand peak at *m*/*z* 546, a peak at *m*/*z* 347 was obtained by loss of the bispicolylamine branch. In

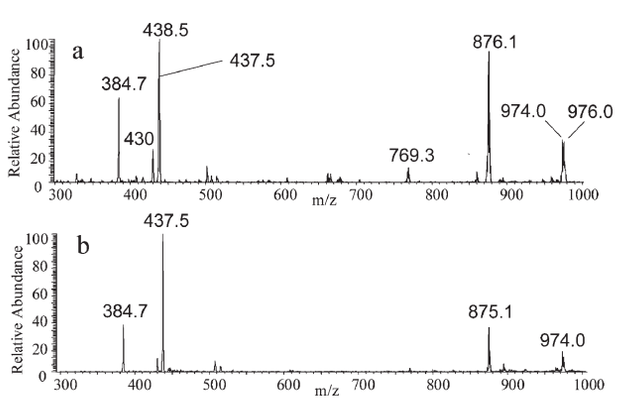


Fig. 1 ESI-MS monitoring of the reaction in acetonitrile of [Fe^{II}Fe^{III}-(L)(mpdp)(CH$_3$CN)]$^{2+}$ (0.2 mM) with 4 equiv. *m*-CPBA after *ca.* 5 s (a) and 5 min (b).

† Electronic supplementary information (ESI) available: ESI-MS of **1** with theoretical isotopic pattern. Multiple-stage tandem ESI-MS analysis of the ligand recovered after extraction of the iron. ^{1}H NMR spectra in CD$_3$CN of [**1**]$^{2+}$ under different conditions and [**2**]$^{+}$. See http://www.rsc.org/suppdata/nj/b4/b402403f/

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contrast, fragmentation of the original ligand (HL^+, m/z 530) under the same conditions gave a peak at m/z 331. This showed that the oxygenation had occurred on the picolylbenzylamine branch. Further fragmentation showed that the picolyl group had not been modified, therefore proving that the benzyl group had been oxygenated.

To more precisely determine the site of oxygenation, the reaction was performed in deuterated acetonitrile and analyzed by ^{1}H NMR. The spectrum of the crude reaction mixture (Fig. S3 in the ESI) showed a set of hyperfine-shifted resonances from −100 to 600 ppm characteristic of $Fe^{II}Fe^{III}$ species together with a broad signal at *ca.* 90 ppm typical of $Fe^{III}Fe^{III}$ complexes of this series.[9,13] Reduction of the reaction mixture with sodium iodide suppressed the broad signal of the $Fe^{III}Fe^{III}$ species and the resulting enhanced spectrum showed the characteristic upfield resonances of the mixed-valent complex of the ligand H(L − H + O), $[Fe^{II}Fe^{III}(L - H + O)(mpdp)]^+$, which bears an *ortho*-hydroxybenzyl group in place of the benzyl.[13] In agreement with the ESI-MS results, traces of another complex were also observed while $[Fe^{II}Fe^{III}(L)(mpdp)(CH_3CN)]^{2+}$ was completely consumed. It appears, therefore, that the benzyl group is *ortho*-hydroxylated by the peracid at a level approaching 90% (based on ^{1}H NMR) within a few minutes. The same transformation can be brought about by the iodosyl arene ArIO but in this case the reaction is far slower, requiring 24 h.

The mechanism of the reaction was investigated by ESI-MS using ^{18}O isotopic labelling under various time and temperature conditions. The key mechanistic observations are summarized in the following. (i) The $[Fe^{II}Fe^{III}(L - H + O)(mpdp)]^+$ peak at m/z 875 developed at the expense of those of $[Fe^{III}Fe^{III}(L - H + O)(mpdp)]^{2+}$ and $[Fe^{III}Fe^{III}(L - H + O)(mpdp)(ClO_4)]^+$ at m/z 437.5 and 974 as the solution was left standing. This indicates that the mixed-valent species $[Fe^{II}Fe^{III}(L - H + O)(mpdp)]^+$ is not the primary product of the reaction. The latter is therefore the diferric complex $[Fe^{III}Fe^{III}(L - H + O)(mpdp)]^{2+}$. (ii) The presence or absence of dioxygen had no effect on the reaction. Consistently, essentially no incorporation of the ^{18}O label was detected when the reaction is run under $^{18}O_2$. These observations indicate that the reaction does not involve a radical mechanism. (iii) When the reaction was performed at room temperature in the presence of 2000 equiv. $H_2{}^{18}O$, a partial incorporation (17%) of ^{18}O into $[Fe_2(L - H + O)(mpdp)]$ was observed [Fig. 2(a)], as shown by the increase of the peak at m/z 438.5 (peak height 36% *vs.* 17.3% in the absence of labelling). (iv) When the reaction was run at −40 °C, the hydroxylation reaction was blocked and two peaks were detected at m/z 876 and 438.5, which moved to 878 and 439.5 in the presence of $H_2{}^{18}O$. These peaks are thus attributed to the oxo- and hydroxo-$Fe^{III}Fe^{III}$ species $[Fe^{III}Fe^{III}(L)(mpdp)(O)]^+$ and $[Fe^{III}Fe^{III}(L)(mpdp)(OH)]^{2+}$, respectively. It is worth noting that these peaks were observed also at short times when the reaction was run at room temperature (spectrum a in Fig. 1). These experiments show that diferric complexes are formed at the early stage of the reaction. (v) When this low-temperature reaction mixture, with excess $H_2{}^{18}O$, was left standing for *ca.* 30 min the peaks of the oxo and hydroxo complexes were replaced by that of the *m*-chlorobenzoate $[Fe^{III}Fe^{III}(L)(mpdp)(m\text{-}ClC_6H_4CO_2)]^{2+}$ at m/z 517. In this process, the ^{18}O label was completely lost. Upon thawing to room temperature in the presence of 4 equiv. *m*-CPBA and 2000 equiv. $H_2{}^{18}O$, the hydroxylation occurred and the hydroxylated product incorporated 8% of the ^{18}O label. Therefore, it appears that incorporation of the ^{18}O label occurs in the final stage of the reaction. (vi) The presence of benzoate must be avoided to stabilize the oxo complex. Accordingly, it was prepared from an iodosyl arene at −40 °C and labelled with $H_2{}^{18}O$ up to 73% [Fig. 2(b)]. Upon pouring this low-temperature solution into a 4 equiv. *m*-CPBA/2000 equiv. $H_2{}^{18}O$ acetonitrile solution at room temperature, the hydroxylation occurred and the product incorporated 53% of the ^{18}O label, as shown in Fig. 2(c). Overall, the hydroxylated product retains 73% of the ^{18}O label of the starting oxo complex.

The above observations allow basic mechanistic features to be delineated. Firstly, owing to the fact that the oxygen transfer is a two-electron process, two reaction pathways can be considered to lead from the mixed-valent complex $[Fe^{II}Fe^{III}(L)(mpdp)]^{2+}$ to the diferric hydroxylated product $[Fe^{III}Fe^{III}(L - H + O)(mpdp)]^{2+}$: (i) formation of an active $Fe^{III}Fe^{IV}$ intermediate giving the hydroxylated product at the $Fe^{II}Fe^{III}$ state ($[Fe^{II}Fe^{III}(L - H + O)(mpdp)]^+$) and its further oxidation to $Fe^{III}Fe^{III}$ or, alternatively, (ii) initial oxidation to a diferric species (Scheme 2, reaction a) and formation of an active $Fe^{IV}Fe^{IV}$ intermediate (Scheme 2, reaction b), which gives rise to the hydroxylated product at the $Fe^{III}Fe^{III}$ state (Scheme 2, reaction c). Since the primary product of the hydroxylation is the diferric complex $[Fe^{III}Fe^{III}(L - H + O)(mpdp)]^{2+}$, and not the mixed-valent analog $[Fe^{II}Fe^{III}(L - H + O)(mpdp)]^+$, *pathway (ii) and a $Fe^{IV}Fe^{IV}$ intermediate must be involved.*

This conclusion is supported by the detection of an oxo-diferric intermediate in the early stages of the room temperature reaction, which could be stabilized at −40 °C. Most probably the oxo ligand is bridging rather than terminal, owing to the ubiquity of μ-oxo-diferric species.[14] Whatever the reaction conditions [experiments (iii), (v) and (vi) above], ^{18}O incorporation was observed in the presence of $H_2{}^{18}O$. *This provides indirect but compelling evidence that an oxo complex is the active species*.[15] Moreover, transfer of the oxo ligand of the μ-oxo-diferric species after its oxidation to $Fe^{IV}Fe^{IV}$ was shown by the high retention of the ^{18}O label in experiment (vi). Nevertheless, part of this ^{18}O label was lost during the thawing process in this experiment. Furthermore, when starting from a non-oxo diferric intermediate as in experiment (v), the incorporation of the ^{18}O label was small. Both observations suggest that two different oxo ligands can be transferred from the active oxo-$Fe^{IV}Fe^{IV}$ species. As a consequence, the latter could be a dioxo-$Fe^{IV}Fe^{IV}$ species, either (di-μ-oxo)- or an (oxo)(μ-oxo)-$Fe^{IV}Fe^{IV}$. Further experiments are needed to distinguish between these two structures. These mechanistic hypotheses are illustrated in Scheme 2.

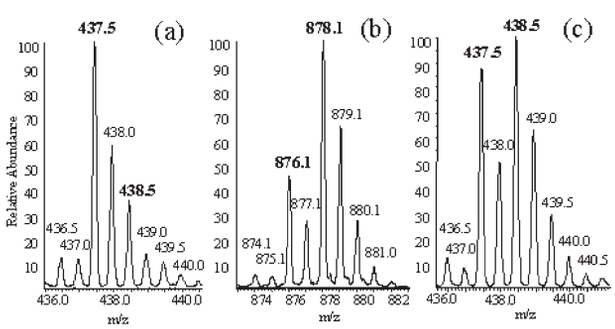

Fig. 2 ESI-MS spectra of the reaction of $[Fe^{II}Fe^{III}(L)(mpdp)(CH_3CN)]^{2+}$ in acetonitrile: (a) with 4 equiv. *m*-CPBA in the presence of 2000 equiv. $H_2{}^{18}O$ at room temperature, (b) with 4 equiv. ArIO in the presence of 2000 equiv. $H_2{}^{18}O$ at −40 °C and (c) 5 min after pouring solution (b) into an acetonitrile solution of 4 equiv. *m*-CPBA/2000 equiv. $H_2{}^{18}O$ at room temperature.

$$[Fe^{III}(L)Fe^{II}\text{-}S]^{2+} \xrightarrow{m\text{CPBA}} [Fe^{III}(L)(\mu\text{-}O)Fe^{III}]^+ \quad \textbf{(a)}$$

$$[Fe^{III}(L)(\mu\text{-}O)Fe^{III}]^+ \xrightarrow{m\text{CPBA}} [Fe^{IV}(L)(\mu\text{-}O)Fe^{IV}(O)]^+ \quad \textbf{(b)}$$

$$[Fe^{IV}(L)(\mu\text{-}O)Fe^{IV}(O)]^+ \longrightarrow [Fe^{III}(L\text{-}H\text{+}O)Fe^{III}]^{2+} \quad \textbf{(c)}$$

Scheme 2 S = acetonitrile.

In summary, we have reported that the mixed-valent $Fe^{II}Fe^{III}$ complex of a phenolate ligand is able to hydroxylate almost quantitatively a dangling benzyl residue of the ligand to give an *ortho*-hydroxybenzyl group at the expense of an oxygen donor. With *m*-CPBA, labelling experiments suggest that the active species is an oxo-$Fe^{IV}Fe^{IV}$ entity. This reactivity bears a strong resemblance to the toluene hydroxylation to *ortho*-cresol performed by toluene-2-monooxygenase,[5] which is structurally similar to methane monooxygenase.[3] Experiments are under way to evaluate the potentiality of this system to oxygenate aromatic and aliphatic substrates and to further investigate the mechanisms of these reactions.

Experimental

$[Fe^{II}Fe^{III}(L)(mpdp)(H_2O)](ClO_4)_2$ (formula: $C_{46}H_{48}N_5O_{14}Cl_2Fe_2$) was prepared as previously described.[9,10] ^1H NMR experiments were performed on a Bruker 200 MHz spectrometer as described earlier.[13] In a typical oxygenation experiment, 4 equiv. of an acetonitrile solution of oxygen donor XO was added to a 0.2 mM acetonitrile solution of $[Fe^{II}Fe^{III}(L)(mpdp)(H_2O)](ClO_4)_2$. For labelling experiments 2000 equiv. of $H_2{}^{18}O$ were added with the oxygen donor. The reaction was monitored by injecting at fixed time intervals into an ESI-MS Finnigan Thermoquest mass spectrometer, equipped with an ion trap and an octupolar analyzer, set up at fixed optimized conditions: source voltage 4.75 kV, capillary voltage 34.84 V, capillary temperature 160.4 °C and flow rate 25 μL min^{-1}. The extent of labelling was calculated from comparison of the respective heights of the peaks at M and M + 2, taking into account their respective contributions at M + 2 (17.6%) and at M (12.6%).

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