# A diiron complex mediates an intramolecular aliphatic hydroxylation by various oxygen donors†

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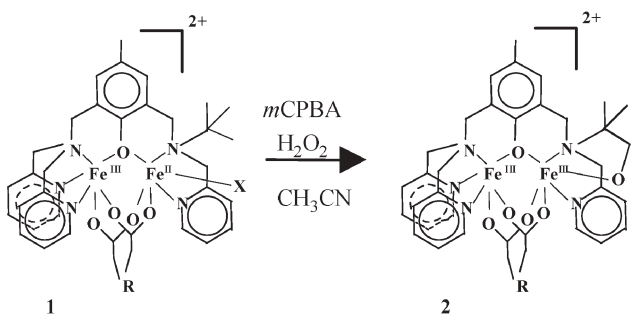
*Received (in Cambridge, UK) 23rd August 2004, Accepted 29th October 2004*
*First published as an Advance Article on the web 2nd December 2004*
**DOI: 10.1039/b412929f**

**In the presence of hydrogen peroxide, *m*-chloroperbenzoic acid or an iodosyl arene, the *tert*-butyl group of the ligand H(L–*t*-Bu) in the complex $[Fe_2(L–t\text{-}Bu)(mpdp)]^{2+}$ is quantitatively hydroxylated to a butanolate terminally bound to one iron in $[Fe_2(L–t\text{-}Bu - H + O)(mpdp)]^{2+}$, and mass spectrometry experiments indicate that the reaction proceeds according to different mechanisms.**

Non-heme iron oxygenases have been attracting a great deal of interest over the last ten years owing to their involvement in the biosynthesis of numerous important biomolecules as well as the detoxification of exogenous substances. These activities very often rely on aliphatic and aromatic hydroxylations[1,2] which are performed by hydroxylases with both mononuclear (*e.g.* phenylalanine hydroxylase[3]) and dinuclear (*e.g.* methane monooxygenase[4]) active sites. Understanding their mechanisms of action and mimicking their activities is a challenging task and only a few model complexes have been reported to perform aromatic[5–7] and aliphatic[8–10] hydroxylations when treated with dioxygen under reducing conditions or with an oxygen donor such as $H_2O_2$, an alkylhydroperoxide or an iodosyl arene. In this communication, we present evidence that $[Fe^{II}Fe^{III}(L–t\text{-}Bu)(mpdp)](BPh_4)_2$, **1** (Scheme 1, mpdp = *m*-phenylenedipropionate) bearing a dangling *tert*-butyl group mediates the hydroxylation of the aliphatic residue at the expense of an oxygen donor (*e.g.* *m*-chloroperbenzoic acid (*m*-CPBA), an iodosyl arene (ArIO) or $H_2O_2$). In addition, $^{18}O$ labelling studies show that this hydroxylation occurs through different mechanisms which depend on the oxygen donor and differ from that observed for the recently reported *o*-hydroxylation of the mixed-valent complex $[Fe^{II}Fe^{III}(L–Bn)(mpdp)(H_2O)](ClO_4)_2$ bearing a dangling benzyl group.[11]




**Scheme 1** (R = 1,3-xylyl; X = acetonitrile)

† Electronic supplementary information (ESI) available: Fig. S1: ESI-MS of **1** and **2**. Fig. S2: multiple stage tandem ESI-MS analysis of the ligand recovered after extraction of the iron. See http://www.rsc.org/suppdata/cc/b4/b412929f/

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**1** was prepared according to usual procedures[12] and characterised by elemental analysis, electrospray ionisation mass spectrometry (ESI-MS) and $^1H$ NMR (supplementary information†). When a 0.2 mM acetonitrile solution of **1** was reacted at room temperature with 4 equivalents of *m*-CPBA, ArIO or $H_2O_2$, the initially blue solution turned dark violet within 5 to 30 min, depending on the oxygen donor.[13] The reaction mixture was analysed by ESI-MS. Fig. S1 illustrates the ESI-MS spectrum of an acetonitrile solution of **1** which presents a major peak at *m*/*z* 826 associated with the monocation $[Fe^{II}Fe^{II}(L–t\text{-}Bu)(mpdp)]^+$ in addition to a peak at *m*/*z* 413 associated with **1** $[Fe^{II}Fe^{III}(L–t\text{-}Bu)(mpdp)]^{2+}$. The reduction of the mixed valent species within the mass spectrometer was already observed and is made easier by the presence of the tetraphenylborate anion. Upon reaction with $H_2O_2$ (or *m*-CPBA or ArIO), these peaks were replaced by new ones at *m*/*z* 420.5 and 841 (Fig. S1†), which correspond to the addition of an oxygen and the loss of a hydrogen leading to $[Fe_2(L–t\text{-}Bu - H + O)(mpdp)]^{2+}$, as expected for the transformation of the $C(CH_3)_3$ group into $C(CH_3)_2(CH_2OH)$ and its coordination to the iron in the corresponding alcoholato complex. The transformation could be monitored by ESI-MS as illustrated in Fig. 1 which shows that the peak associated with **1** at *m*/*z* 413 is progressively replaced by a new one at *m*/*z* 420.5 associated with $[Fe^{III}Fe^{III}(L–t\text{-}Bu - H + O)(mpdp)]^{2+}$, **2**. The latter compound is very prone to reduction to the mixed valent monocation (identified by its peak at *m*/*z* 841) within the spectrometer or upon standing in solution owing to the presence of the tetraphenylborate anion.

The nature of the oxygenated product was confirmed by a multiple stage tandem ESI-MS analysis of the ligand recovered after extraction of the iron ions which located the hydroxylation on the *t*-Bu residue. Indeed after extraction of the iron, the ligand was recovered and shown to exhibit a peak at *m*/*z* 512 associated

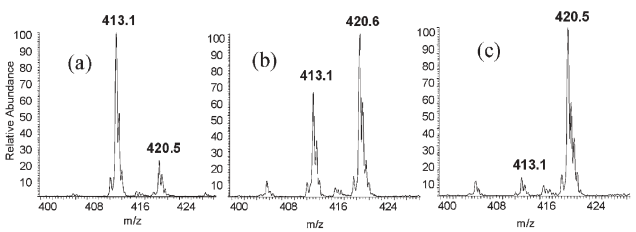



**Fig. 1** ESI-MS monitoring of the solution 2 min (a), 10 min (b) and 20 min (c) after the reaction at room temperature of **1** with 4 equiv. of $H_2O_2$.

to $H_3(L–t\text{-}Bu - H + O)^+$, as opposed to *m*/*z* 496 for $H_2(L–t\text{-}Bu)^+$. Two successive fragmentations of $H_3(L–t\text{-}Bu - H + O)^+$ led to the loss of the $C(CH_3)_2(CH_2OH)$ residue (Fig. S2†).

The mechanisms of this transformation were investigated by ESI-MS using $^{18}O$ labelled reagents. As shown in Fig. 2a, when **1** was reacted with $H_2O_2$ in the presence of $^{18}O_2$, the ESI-MS of **2** was dominated by a peak at *m*/*z* 843 pointing to the incorporation of 72(4)% of oxygen from $O_2$. On the other hand, when **1** was reacted with $H_2{}^{18}O_2$ in air, the ESI-MS of **2** was dominated by a peak at *m*/*z* 841 pointing to the incorporation of 86(4)% of oxygen from $O_2$. Interestingly, when the latter reaction was performed under argon, the ESI-MS spectrum showed that **2** had incorporated 90(4)% of $^{18}O$ (Fig. 2c). These experiments show that two competitive mechanisms operate for the aerobic hydroxylation of the dangling *t*-Bu residue in **1**. Indeed, *ca.* 20% of the inserted oxygen comes from hydrogen peroxide while *ca.* 80% comes from $O_2$, suggesting that oxygenation by $H_2O_2$ is dominated by a radical pathway (Scheme 2).

The same kinds of experiments were performed with *m*-CPBA as the oxygen donor. When the reaction was run in the presence of $^{18}O_2$, ESI-MS analysis revealed insignificant levels (<2%) of label incorporation. When the reaction was run at −40 °C, a complicated mass spectrum was observed showing the presence of peaks at *m*/*z* 420.5 and 421.5 in the dication domain, and at *m*/*z* 841 and 842 for the monocations. In the presence of $H_2{}^{18}O$, the peaks at 421.5 and 842 moved to 422.5 and 844, but those at 420.5 and 841 were unaffected. The peaks at 421.5 and 842 can be assigned to the hydroxo and oxo complexes $[Fe^{III}Fe^{III}(L–t\text{-}Bu)(mpdp)(OH)]^{2+}$ and $[Fe^{III}Fe^{III}(L–t\text{-}Bu)(mpdp)(O)]^+$, which is consistent with the present labelling experiments and earlier observations.[11] It is worth noting that the hydroxylated product appearing at *m*/*z* 420.5 and 841 did not incorporate the $^{18}O$ label. Interestingly, when the same experiments were performed with the iodosyl arene, the same behaviour was observed except for the significant (19(4)%) incorporation of the $^{18}O$ label (Scheme 2).

These experiments allowed the main steps of the mechanisms to be delineated. The reaction of **1** with either *m*-CPBA or ArIO involves the initial formation of the oxo complex $[Fe^{III}Fe^{III}(L–t\text{-}Bu)(mpdp)(O)]^+$ which is protonated to $[Fe^{III}Fe^{III}(L–t\text{-}Bu)(mpdp)(OH)]^{2+}$ as already observed for the complexes of the benzyl ligand.[11] Nevertheless, in the latter case, the oxygenation of the benzyl group of the ligand by *m*-CPBA was blocked at −40 °C and occurred at higher temperatures with incorporation of the $^{18}O$ label from $H_2{}^{18}O$ providing indirect but compelling evidence that it involves a high-valent Fe=O intermediate.[11] In the case of **1** with *m*-CPBA the absence of $^{18}O$ label incorporation from $H_2{}^{18}O$ does not strictly exclude an oxoiron intermediate if its exchange with water is slower than the oxygen transfer. This hypothesis is supported by the observation that the hydroxylation of the *t*-Bu group is faster than that of the benzyl. Nevertheless, it cannot be excluded that the species active in the oxygenation may be the peracid adduct.[14] No conclusion can be derived for ArIO since it exchanges its oxygen with water at a rate comparable to that of the oxygenation. In any case, a radical pathway is excluded by the absence of labelling with $^{18}O_2$.

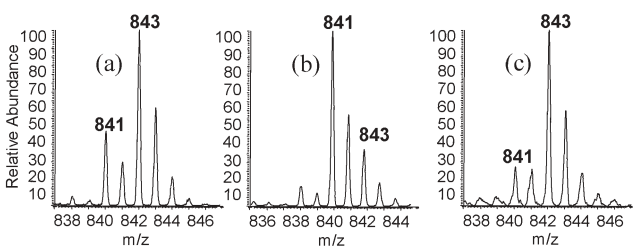
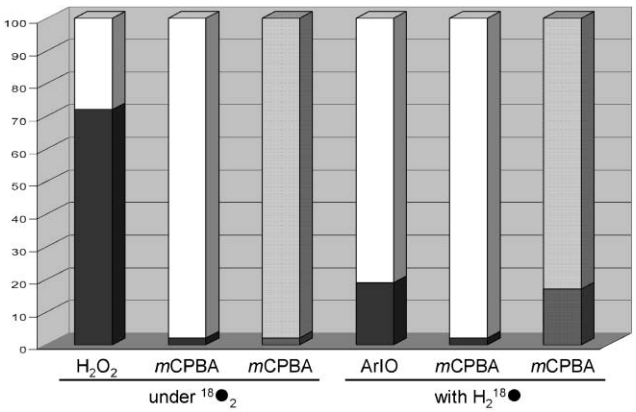



**Fig. 2** ESI-MS spectrum of the solution after the reaction at room temperature of **1** (a) with 4 equiv. of $H_2{}^{16}O_2$ under $^{18}O_2$, (b) with 4 equiv. of $H_2{}^{18}O_2$ in air, and (c) with 4 equiv. of $H_2{}^{18}O_2$ under argon.




**Scheme 2** Extent of labelling (black filling) of **2** and the benzyl analog (crossed bars) under labelling conditions with $^{18}O_2$ and $H_2{}^{18}O$.

The mechanism of oxygenation with hydrogen peroxide differs strongly since 80% of the reaction follows a radical pathway. The reaction of $H_2O_2$ with $[Fe^{II}Fe^{III}(L–Bn)(mpdp)(H_2O)](ClO_4)_2$ was shown to give the corresponding hydroxo and hydroperoxo complexes[15] as observed for mononuclear iron(II) complexes.[8] The homolytic cleavage of the hydroperoxide is the simplest way to explain the *t*-Bu hydroxylation since abstraction of an H atom by $HO^{\bullet}$ would generate a radical on the substrate and therefore explain the radical pathway after trapping by dioxygen. On the other hand, recombination of the radical with the $Fe^{IV}$=O species would account for the incorporation of oxygen from hydrogen peroxide into the hydroxylated product. Overall, the reactivity of the present complex with hydrogen peroxide is quite similar to that observed for some mononuclear iron complexes[8,16] and therefore it appears that the ferric ion in the bispicolylamine site may not play any role in the oxygen transfer. This is at odds with its behaviour in the *m*-CPBA mediated oxygenation of the benzyl group which is believed to involve the oxidation of both iron ions leading to an oxo-diiron(IV) species achieving a non-radical oxygen transfer.[11] It is striking that while the dinuclear nature of the complexes is enforced by the built-in bridging phenolate, the observed reactivity is akin to that of mononuclear compounds. This indicates that even though the overall reactivity is the same for all oxygen donors, hydrogen peroxide, as used in the *peroxide shunt*, may operate along a specific mechanism, totally different in particular from enzymatic ones.

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## Notes and references


1 E. Y. Tshuva and S. J. Lippard, *Chem. Rev.*, 2004, **104**, 987.
2 M. Costas, M. P. Mehn, M. P. Jensen and L. Que, Jr, *Chem. Rev.*, 2004, **104**, 939.
3 T. Flatmark and R. C. Stevens, *Chem. Rev.*, 1999, **99**, 2137.
4 M.-Y. Baik, M. Newcomb, R. A. Friesner and S. J. Lippard, *Chem. Rev.*, 2003, **103**, 2385.
5 S. Ménage, J. B. Galey, G. Hussler, M. Seité and M. Fontecave, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2353.
6 S. Mukerjee, A. Stassinopoulos and J. Caradonna, *J. Am. Chem. Soc.*, 1997, **119**, 8097.
7 M. P. Jensen, S. J. Lange, M. P. Mehn, E. L. Que and L. Que, Jr, *J. Am. Chem. Soc.*, 2003, **125**, 2113.
8 K. Chen and L. Que, Jr, *J. Am. Chem. Soc.*, 2001, **123**, 6327.
9 T. L. Foster and J. P. Caradonna, *J. Am. Chem. Soc.*, 2003, **125**, 3678.
10 J. Kaizer, E. J. Klinker, N. Y. Oh, J.-U. Rohde, W. J. Song, A. Stubna, J. Kim, E. Münck, W. Nam and L. Que, Jr, *J. Am. Chem. Soc.*, 2004, **126**, 472.
11 F. Avenier, L. Dubois and J.-M. Latour, *New J. Chem.*, 2004, **28**, 782.
12 S. Chardon-Noblat, O. Horner, B. Chabut, F. Avenier, N. Debaecker, P. Jones, J. Pécaut, L. Dubois, C. Jeandey, J.-L. Oddou, A. Deronzier and J.-M. Latour, *Inorg. Chem.*, 2004, **43**, 1638.
13 In a typical oxygenation experiment, 4 equiv. of a solution of the oxygen donor were added to a 0.2 mM acetonitrile solution of **1b**. For labelling experiments 90% $^{18}O$ enriched $H_2{}^{18}O_2$ (2.2% aqueous solution, Leman Co, USA), 96% $^{18}O$ enriched $^{18}O_2$ (Eurisotop, France) and 95% $^{18}O$ enriched $H_2{}^{18}O$ (Eurisotop, France) were used. The reaction was monitored by injection into an ESI-MS Finnigan Thermoquest mass spectrometer, equipped with an ion trap and an octupolar analyser, set up at fixed optimised 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%).
14 W. Nam, M. H. Lim, S. K. Moon and C. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 10805.
15 F. Avenier, L. Dubois, P. Dubourdeaux and J.-M. Latour, unpublished observations.
16 M. P. Jensen, M. P. Mehn and L. Que, Jr, *Angew. Chem., Int. Ed.*, 2003, **42**, 4357.