ChemComm

Deprotonation of low-spin mononuclear iron(III)–hydroperoxide complexes give transient blue species assigned to high-spin iron(III)–peroxide complexes

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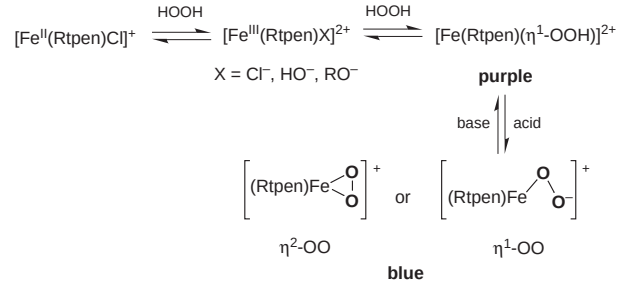
Received (in Cambridge, UK) 14th April 1999, Accepted 24th May 1999

Purple iron(III)–hydroperoxide complex ions [Fe(Rtpen)(η^1-OOH)]$^{2+}$ can be reversibly deprotonated to give transient blue species showing spectroscopic properties transient with iron(III)–peroxide complexes; a novel ferryl species is produced in MS/MS experiments with the iron(III) hydroperoxide ion.

Dioxygen activation chemistry by a single iron atom occurs in some non-heme iron enzymes[1] and the antitumor drug bleomycin (BLM).[2] The characterisation of model mononuclear non-heme Fe(III)–peroxide species is expected to aid the structural elucidation of similar species in the catalytic cycles of these non-heme iron biomolecules. In recent years several biologically relevant transient mononuclear non-heme iron(III)–hydroperoxide model systems have been identified in solution.[3–5] These species are proposed to contain end-on (η^1) hydroperoxide ligands. The only mononuclear non-heme complex proposed to contain a side-on (η^2) peroxide ligand is [Fe(edta)O_2].[6,7]

A mononuclear iron(II) complex of the pentadentate ligand *N*-methyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)ethane-1,2-diamine (metpen) [Fe(metpen)Cl](PF_6) when treated with an excess of H_2O_2 in hydroxylic solvents generates purple solutions.[3] The UV-VIS and EPR spectra of this solution show features comparable to other reported Fe(III)–η^1-OOH systems[5] and bleomycin,[2] supporting formulation of the transient purple species as the low-spin Fe(III)–hydroperoxo compound [Fe(metpen)(η^1-OOH)]$^{2+}$. By carrying out simple ligand modifications of the parent metpen ligand we have continued this work towards the ultimate aim of solid state characterisation, and we communicate here the spectroscopic identification of new unique mononuclear Fe(III)–peroxide species obtained by deprotonation of the hydroperoxide species [Fe(Rtpen)(η^1-OOH)]$^{2+}$, where Rtpen is the generic abbreviation for the ligand series; *N*-ethyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)ethane-1,2-diamine (ettpen) and *N*-benzyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)-ethane-1,2-diamine (bztpen).

The yellow crystalline iron(II) and iron(III) complexes [Fe(Rtpen)Cl]A and [Fe(Rtpen)Cl]$(A)_2$ (A = ClO_4 or PF_6), are prepared by the reaction of each ligand with either iron(II) or iron(III) chloride in dry methanol followed by addition of a salt of the counter anion. Crystal structures of the iron(II) compounds have been obtained. The iron(III) species are not stable in solution: their reduced counterparts and orange iron(III) compounds, formulated as [(Rtpen)ClFe(μ-O)FeCl(Rtpen)]$(A)_2$, can be isolated from aged solutions of [Fe(Rtpen)Cl]$(A)_2$. The reactions of the iron(II) and iron(III) compounds with an excess of hydrogen peroxide in hydroxylic solvents generate purple solutions which are stable for hours. There is a time lag of a few seconds before the appearance of the purple colour when the iron(II) starting materials are used. This time lag supports the notion of a rate-determining formation of an intermediate low-spin iron(III) compound (detected by EPR) before a ligand exchange reaction with deprotonated hydroperoxide. A possible reaction scheme is presented in Scheme 1. By using the iron(III) starting materials [Fe(bztpen)Cl]$(A)_2$ (A = ClO_4 or PF_6), formation of the hydroperoxide species is expedited since formation of the purple colour is, in these cases, instantaneous. The purple chromophores are proposed to be the low-spin Fe(III)–hydroperoxide complexes containing end-on bound hydroperoxide ligands, [Fe(Rtpen)(OOH)]$^{2+}$ with spectroscopic characteristics similar to those we reported[3] for [Fe(metpen)(OOH)]$^{2+}$. The addition of ammonia, triethylamine or pyridine to the purple solutions produces a transient blue colour. If the base is first added to methanolic solutions of [Fe(Rtpen)Cl]$^+$ followed by hydrogen peroxide the blue solution is generated directly. Subsequent addition of hydrochloric acid to the blue solutions regenerates the purple solution. This cycle can be repeated several times with the same solution indicating a reversible acid/base equilibrium. We assign the blue species to a novel iron(III)–peroxide compound derived from the deprotonation of the purple [Fe(Rtpen)(OOH)]$^{2+}$. The spectroscopic data obtained so far is consistent with a mononuclear high-spin iron(III) complex with either side-on (η^2) or end-on (η^1) peroxide coordination (Scheme 1).



Scheme 1

UV-VIS spectra for [Fe(ettpen)Cl]PF_6 and its proposed purple Fe(III)–hydroperoxide and blue iron(III)–peroxide derivatives are shown in Fig. 1. Similar data were obtained for the other members of the series.[8] The difference in the maxima assigned to the peroxide to iron(III) charge-transfer band in the purple and blue species is consistent with expected increased donor strength of the O_2^{2-} *vs.* O_2H^- ligand. However this simple argument is complicated by a concomitant spin change of the iron atom (see EPR results below).

Peaks assigned to both the complexes, [Fe(bztpen)(OOH)]$^+$ and [Fe(bztpen)(OO)]$^{2+}$ are observed in the ESI mass spectra of the purple solutions (Fig. 2). Consistent with the proposed acid/base equilibrium the ratio between *m/z* 511.2 and 256.1 ions increases in spectra of the blue solutions (*m/z* 511.2 is weak in Fig. 2). A prominent peak (often the most intense) at *m/z* 247.6 can be assigned to the ferryl species, [Fe(bztpen)O]$^{2+}$. Collision induced dissociation (CID) of the ion at *m/z* 256.1 produces ions at *m/z* 247.6, 239.6 and 194.6. The first two of these ions are assigned to the ferryl species [Fe(bztpen)O]$^{2+}$ and a ferrous species [Fe(bztpen)]$^{2+}$, and explained by loss of a hydroxyl and hydrosuperoxide radical, respectively, from [Fe(bztpen)(OOH)]$^{2+}$. The third peak is assigned to [Fe(bztpen − C_7H_6)]$^{2+}$. In order to determine whether or not the peaks at *m/z* 239.6 and 194.6 could be the result of the decomposition of the *m/z* 247.6

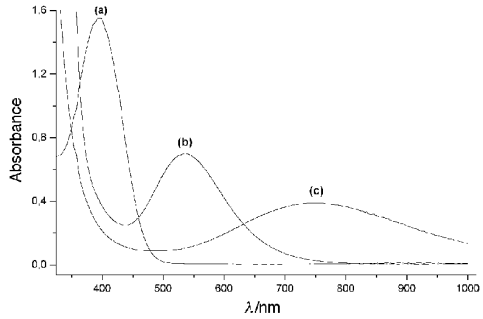


Fig. 1 UV-VIS spectra of (a) 7.5×10^{-4} M [Fe(ettpen)Cl]PF_6 in MeOH, (b) after addition of 300 equiv. of H_2O_2 and followed by (c) addition of 35 equiv. of Et_3N.

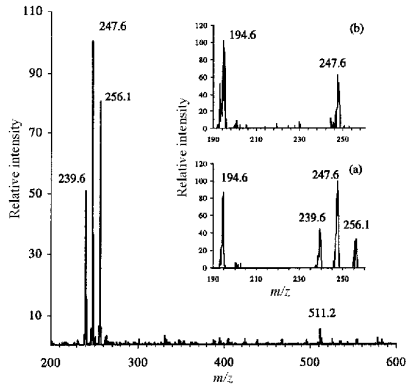
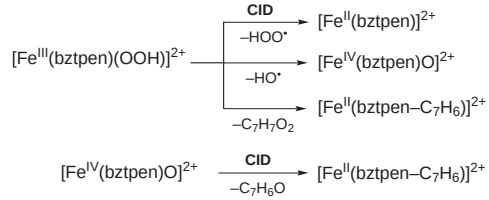


Fig. 2 The ESI mass spectrum of the purple solution generated by the reaction of [Fe(bztpen)Cl]$(ClO_4)_2$ with 100 equiv. of H_2O_2 in ethanol. Inserts show the CID spectra of the (a) *m/z* 256.1 and (b) *m/z* 247.6 ions. Assignments: *m/z* 256.1, [Fe(bztpen)(OOH)]$^{2+}$; 511.2, [Fe(bztpen)(OO)]$^{+}$; 247.6, [Fe(bztpen)O]$^{2+}$; 239.6, [Fe(bztpen)]$^{2+}$; 194.6, [Fe(bztpen − C_7H_6)]$^{2+}$.

ion {[Fe(bztpen)O]$^{2+}$} as well as, or rather than the hydroperoxide ion, [Fe(bztpen)(OOH)]$^{2+}$ a second MS/MS experiment on the *m/z* 247.6 ion was performed. This resulted only in the generation of the *m/z* 194.6 ion. Loss of 106 mass units from [Fe(bztpen)O]$^{2+}$ can be ascribed to loss of the mass equivalent to benzaldehyde, and intramolecular oxo transfer from the ferryl to the dangling benzyl group seems to be a plausible explanation. The iron(II) species which is expected to remain after benzaldehyde loss, [Fe^{II}(bztpen − C_7H_6)]$^{2+}$ (= {[Fe^{IV}(bztpen)O] − C_7H_6O}$^{2+}$) fits with observation of the *m/z* 194.6 ion. In contrast, the CID spectrum obtained under similar conditions of the *m/z* 511.2 ion {[Fe(bztpen)O_2]$^{+}$} does not show any of the peaks assignable to [Fe^{IV}(bztpen)O]$^{2+}$; instead only the ligand decomposition reaction is observed. In summary reaction proceeds *via* Scheme 2.



Scheme 2

The comparative ease of O–O (and Fe–O) bond cleavage in [Fe(metpen)(OOH)]$^{2+}$ compared with [Fe(metpen)(OO)]$^{+}$ observed in the MS/MS experiments is in agreement with the expectation that hydroperoxide species are more reactive (in solution) towards the O–O bond cleavage to give highly reactive ferryl oxidants, compared to their peroxide counterparts. It is for this reason, for example, iron(III) hydroperoxide species are proposed in the DNA degradation process catalysed by bleomycin.[2]

EPR spectra of the purple solutions generated from the reaction of [Fe(bztpen)Cl]PF_6 or [Fe(bztpen)Cl]$(PF_6)_2$ with hydrogen peroxide show overlapping rhombic signals indicative of two low-spin Fe(III) species, their relative intensities depending on the amount of hydrogen peroxide added and the time lag. These results are consistent the formation of [Fe(bztpen)(η^1-OOH)]$^{2+}$ (g = 2.20, 2.16, 1.96) and its precursor, [Fe^{III}(bztpen)X]$^{2+}$, X = Cl^-, OH^- or OR^-, (g = 2.32, 2.14, 1.93) as depicted in Scheme 1. The EPR spectra of blue solutions produced by the addition of base develop new signals at g = 7.60 and 5.74 due to formation of a high-spin iron(III) species. A direct relationship between the concentration of the high-spin species in the EPR spectra and the absorbance of the 748 nm band was verified by recording EPR spectra on a series of frozen solutions in which the blue chromophore showed different concentrations. A calibration of the EPR signals was made.[9]

It is tempting to make a structural assignment of seven coordination for the high-spin blue Fe(III)–peroxide species, *i.e.* [Fe(bztpen)(η^2-OO)]$^{+}$, however other structural alternatives cannot be excluded, *e.g.* [Fe(bztpen)(η^1-OO)]$^{+}$ or six-co-ordinated iron(III) with side-on peroxide and a non-co-ordinated ligand picolyl pendant arm. The possibility that the blue species is a dinuclear μ:η^2,η^2-O_2 species analogous to the dicopper hemocyanin model of Kitajima *et al.*[10] appears to be eliminated since the expected strong antiferromagnetic coupling would cause EPR silence.

In summary, the partial spectroscopic characterisation of three biologically relevant non-heme Fe(III)(O_2H), Fe(III)(O_2) and Fe(IV)O (mass spectrometry only) motifs has been achieved. To our knowledge this is the first observation of the acid–base chemistry of non-heme Fe(III)–peroxo species.

This work was supported by a grant to C. J. M. from the Danish Natural Science research council. We are grateful to a referee for suggesting the EPR calibration and UV-VIS–EPR spectroscopy correlation experiments.

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8 Purple species, λ_{max}/nm: [Fe(metpen)(OOH)]$^{2+}$, 536; [Fe(ettpen)-(OOH)]$^{2+}$, 536: blue species, λ_{max}/nm: [Fe(metpen)(OO)]$^{2+}$, 748; [Fe(ettpen)(OO)]$^{2+}$, 747.
9 The related compounds [Fe(tpen)]$(ClO_4)_3$ [tpen = *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethane-1,2-diamine and Na[Fe(edta)] (edta^{4-} = ethylendiaminetetraacetate) were used to calibrate the low-spin iron(III) and high-spin iron(III) signals, respectively. Possible differences in the rates of relaxation for the various iron compounds were eliminated by calibrating at several probe temperatures. Thus an estimation of the percentage conversion in the reactions with hydrogen peroxide was made. 100% of the iron(II) species [Fe(bztpen)Cl]$^{+}$ can be converted to the low-spin iron(III) species [Fe(bztpen)X]$^{2+}$ and [Fe(bztpen)OOH]$^{2+}$ (relative amounts depending on the H_2O_2 excess); 25% of the low-spin [Fe(bztpen)OOH]$^{2+}$ can be converted to the high-spin signal assigned to [Fe(bztpen)OO]$^{+}$. There are two factors which might be responsible for the apparent incomplete conversion: (i) All the reactions are in equilibrium (as depicted in Scheme 1) and the iron(II) species are favoured. (ii) Decomposition of all or some of the iron(III) species depicted in Scheme 1 and formation of EPR silent oxo-bridged dimers. In support of the second explanation peaks assignable to [(bztpen)Cl-Fe(μ-O)FeCl(bztpen)]$^{2+}$ (*m/z* 522) are indeed observed in ESI mass spectra of the purple and blue solutions.
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Communication 9/02985K