# Characterization of a Nonheme Mononuclear Peroxoiron(III) Intermediate by UV/Vis and EPR Spectroscopy and Mass Spectrometry

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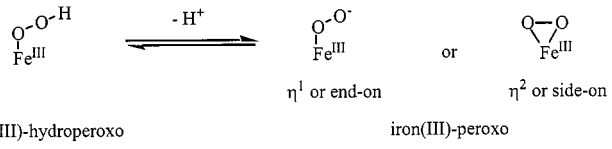
Some nonheme hydroperoxoiron(III) species have been recently characterized by several groups. The reported examples were obtained by adding $H_2O_2$ in excess to an $Fe^{II}$ complex with a neutral polypyridine ligand. We show here that on deprotonation, the purple low-spin hydroperoxoiron(III) complex $[L^5Fe^{III}OOH]^{2+}$ [$L^5$ = *N*-methyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)ethane-1,2-diamine] gives a blue high-spin species which we characterized as the $\eta^2$-peroxoiron(III) species $[L^5Fe^{III}O_2]^+$. It seems that with such an auxiliary ligand, the hydroperoxo group is acidic in contrast with its basicity when it is coordinated to a heme group.

Several nonheme hydroperoxoiron(III) complexes have been recently prepared[1−6] as models of "Activated Bleomycin" which was demonstrated to contain an $Fe^{III}OOH$ unit.[7] A hydroperoxoiron(III) intermediate has also been postulated as an active oxidant in a cytochrome P450 mutant[8][9] and in heme oxygenase.[10] A peroxo intermediate has been detected by Benson et al.[11] and Vaz et al.[12] in cytochrome P450 mutants in which the protonation of the distal oxygen atom of the peroxo group is inhibited. Peroxoiron(III) species have been observed several times in heme models,[13−15] but only once in a nonheme model system.[16] In these models an $\eta^2$-coordination mode was suggested based on spectroscopic studies.




Scheme 1

We show here that, by deprotonation of the hydroperoxoiron(III) species, prepared with the pentadentate ligand $L^5$ [*N*-methyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)ethane-1,2-diamine],[3][6] a peroxoiron(III) species is obtained. EPR spectroscopy suggests a side-on coordination mode for the peroxo group.

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The synthesis and spectroscopic characteristics of $[L^5Fe^{II}Cl]PF_6$ have been reported by Bernal et al.[3] In MeOH, an excess of $H_2O_2$ (100 equiv.) leads to the purple species $[L^5Fe^{III}OOH]^{2+}$.[3][6] It exhibits a UV/Vis spectrum with an intense band at $\lambda_{max}$ = 537 nm ($\varepsilon$ = 1000 $M^{-1}\cdot cm^{-1}$) and an EPR spectrum typical for a low-spin $Fe^{III}$ species ($g_2$ = 2.19, $g_2$ = 2.12, $g_3$ = 1.95). We have synthesized the analogous $[L^5Fe^{II}Cl]Cl$ complex and observed the same intermediate in the presence of $H_2O_2$ which we have also detected by mass spectrometry at *m*/*z* 218.[6]

Upon adding a base to a purple methanol solution of $[L^5Fe^{III}OOH]^{2+}$, a blue color appeared and was stable for about 15 minutes at room temperature. Figure 1 shows the UV/Vis spectra recorded at 0 °C before and after addition of 5 equiv. (based on Fe) of triethylamine. The band at 537 nm disappeared and a new band appeared at 740 nm ($\varepsilon$ = 500 $M^{-1}\cdot cm^{-1}$). This blue species has also been obtained with other bases (NaOH, 2,6-dimethylpyridine). Depending on the p$K_a$, different amounts of base were needed to complete the reaction. To obtain the maximum absorbance of the band at 740 nm, 3 equiv. of NaOH, 5 equiv. of $NEt_3$ or 50 equiv. of 2,6-dimethylpyridine were necessary. Because of its steric hindrance 2,6-dimethylpyridine cannot coordinate to the iron center, confirming that the blue adduct is obtained by deprotonation of $[L^5Fe^{III}OOH]^{2+}$.

The same blue species has also been characterized at 10 K by EPR spectroscopy as shown in Figure 2. Spectra recorded at various temperatures ranging from 4 K to 30 K are typical for high-spin $Fe^{III}$. The analysis of the set of spectra led to the conclusion that two different species were present. One of them has an $E/D$ = 0.29 with the resonances at $g$ = 9.3 and $g$ = 4.3 arising from the lower and middle Kramers doublet of a nearly rhombic complex.[17] The other has an $E/D$ = 0.08 with the resonances at $g$ = 7.5 and 5.9 arising from the upper Kramers doublet and the middle one with $D < 0$.[18] The decrease in intensity of

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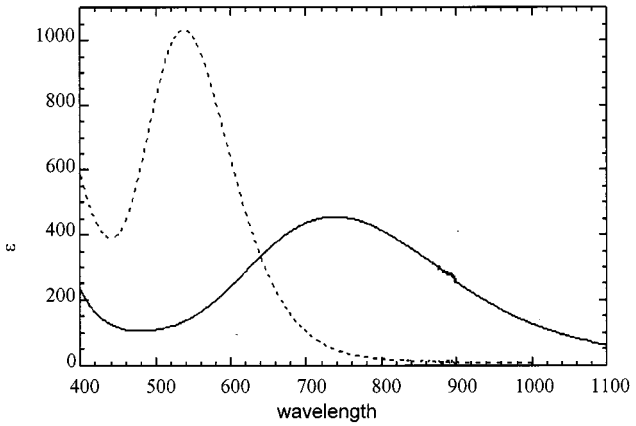



Figure 1. UV/Vis spectra at 0°C of $[L^5Fe^{III}OOH]^{2+}$ (1 mM in MeOH, 100 equiv. of $H_2O_2$) before (- - -) and after addition of 5 equiv. of $NEt_3$ (−); the bands at 537 nm and 740 nm are attributed to LMCT transitions respectively in $[L^5Fe^{III}OOH]^{2+}$ and $[L^5Fe^{III}O_2]^+$; ε in $M^{-1}\cdot cm^{-1}$, wavelength in nm

the UV/Vis band at 740 nm was followed at room temperature as a function of time while EPR spectra were recorded at 10 K on several aliquots sampled at known times and quickly frozen in liquid nitrogen. During the course of the experiment, the resonances at $g = 7.5$ and $g = 5.9$ decreased in intensity while that at $g = 4.3$ increased. The $E/D = 0.08$ species corresponds to the blue intermediate and that at $E/D = 0.29$ to a $Fe^{III}$ degradation product.

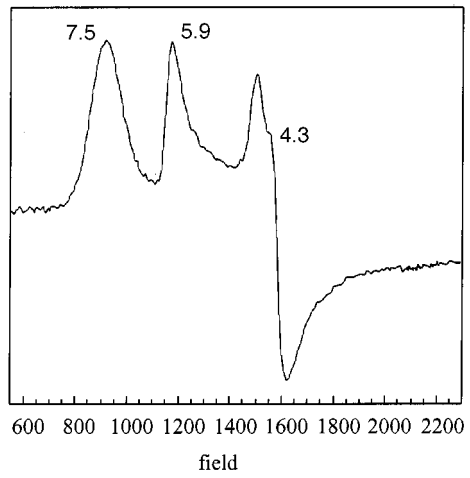



Figure 2. EPR spectrum of $[L^5Fe^{III}OOH]^{2+}$ + 5 $NEt_3$ recorded at 10 K; the resonances at $\delta = 7.5$ and 5.9 are attributed to the quasi-axial high-spin $[L^5Fe^{III}O_2]^+$ species and the resonances at $\delta \approx 4.3$ to a rhombic high-spin $Fe^{III}$ degradation product; field in gauss

Electrospray ionization mass spectra of methanol solutions of [L5FeCl]Cl treated with $H_2O_2$ and either NaOH or triethylamine were identical. Both spectra exhibit a peak at *m/z* 435 (Figure 3) which can be attributed to $[L^5Fe^{III}O_2]^+$. This assignment is supported by the theoretical isotopic pattern shown in Figure 3 and corroborates the fact that the hydroperoxoiron group is acidic enough to yield a per-

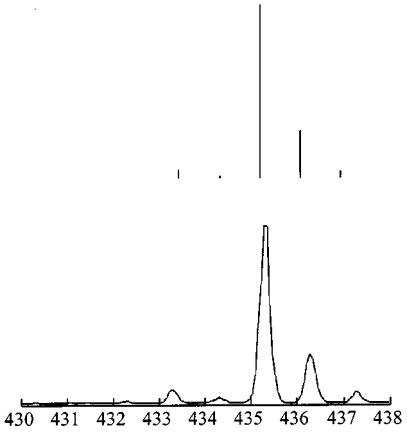
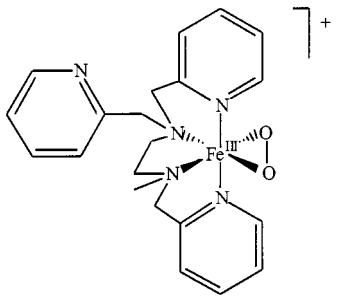



Figure 3. Electrospray-ionization mass-spectroscopic features (*m/z* values) of $[L^5Fe^{III}O_2]^+$ at *m/z* 435; theoretical isotope patterns are represented by bars above the peak cluster

oxoiron species in the presence of a base. We propose that the blue species is the high-spin $Fe^{III}$ species $[L^5Fe^{III}O_2]^+$.

By analogy with $[L^5Fe^{III}OOH]^{2+}$, one expects an $\eta^1$-peroxo complex $[L^5Fe^{III}O_2]^+$, in which $L^5$ is pentacoordinated, to be low spin. The blue species must thus contain a peroxo group linked in an $\eta^2$-mode. Moreover the EPR spectrum of $[L^5Fe^{III}O_2]^+$ is very similar to those of two high-spin iron(III) species with two *cis*-chloro groups: $[(bispicMe_2en)Fe^{III}Cl_2]^+$[19][20] and $[(bispicBz_2en)Fe^{III}Cl_2]^+$.[21] We thus suggest that $[L^5Fe^{III}O_2]^+$ has the structure represented in Scheme 2.



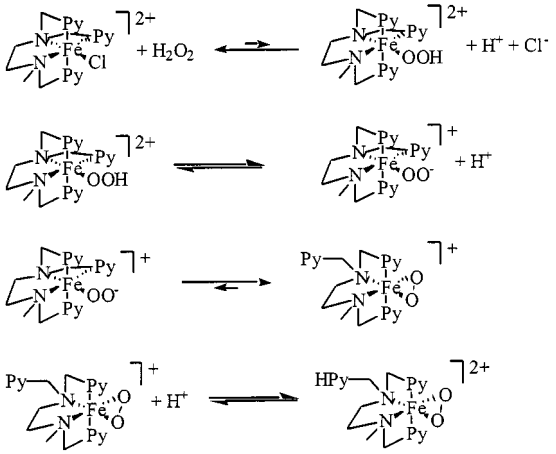

Scheme 2

In this complex, the pentadentate ligand $L^5$ acts as a tetradentate one as already reported by Nivorozhkin et al.[22] for $[L^5ClFe^{III}OFe^{III}ClL^5]^{2+}$. Raman spectroscopy experiments have been performed to observe a peroxidic vibrational signal but the adduct appeared to degrade when irradiated as already described for a similar compound.[2]

The deprotonation of $[L^5Fe^{III}OOH]^{2+}$ has also been studied with a smaller amount of base ($NEt_3/Fe < 5$ or $NaOH/Fe < 3$) by UV/Vis spectroscopy at 2 °C. Under these experimental conditions, the blue species was not formed quantitatively, and moreover it disappeared slowly while the $[L^5Fe^{III}OOH]^{2+}$ species reappeared, but without attaining its original concentration. This slow and incomplete reappearance of $[L^5Fe^{III}OOH]^{2+}$ was attributed to a slow increase in proton concentration due to the sluggish formation of $Fe^{III}-O-Fe^{III}$ decomposition products fol-

lowing the condensation reaction according to Equation 1. The experimental observations reported here can be summarized by the reactions shown in Scheme 3.

$$2\ Fe^{III}(OH_2) \rightarrow Fe^{III}-O-Fe^{III} + H_2O + 2\ H^+ \quad (1)$$



Scheme 3

During the first step, a chloro group of $[L^5Fe^{III}Cl]^{2+}$ is replaced by a hydroperoxo group. As this reaction needs an excess of $H_2O_2$, the reverse reaction is assumed to be predominant. The second step is the acid-base equilibrium between $[L^5Fe^{III}OOH]^{2+}$ and its deprotonated $\eta^1$-form. The next reaction leads to the blue $[L^5Fe^{III}O_2]^+$ adduct after coordination of the peroxo group in an $\eta^2$-fashion and decoordination of a pyridine group. This reaction must be rapid and favorable since we did not detect a low-spin species such as $\eta^1$-$[L^5Fe^{III}O_2]^+$. The protonation of the pendant pyridine moiety is certainly possible. However, this cannot compete since pyridine is a weak base.

In conclusion, our study reports the formation and spectroscopic characterization of a new nonheme mononuclear iron(III) complex with an $\eta^2$-peroxo and a nonoxygenated ligand. In the model presented here, as in other examples of artificial peroxoiron(III) units, the $\eta^2$-coordination mode is found, which must correspond to a profound trend for this unit. Proteins as cytochrome P450 may stabilize the intrinsically unstable $\eta^1$-coordination either by hydrogen bonding with amino acid groups in the distal side or, as proposed recently by Selke and Valentine,[23] by the axial cysteine ligand that may act as a switch that opens the side-on bound peroxo. Our study shows also that the deprotonation of the hydroperoxoiron(III) is thermodynamically easy and may be easier than with heme or bleomycin systems since we are able to produce the peroxo species in the presence of water while the peroxo species of cytochrome P450, for example, is protonated by a water molecule.[24] The acidity or basicity of the hydroperoxo group must be a function of the auxiliary ligand: Negatively charged ligands like hemes must favor basicity and neutral ligands acidity. It is clear that the enhancement of basicity in the case of hemes is crucial for the subsequent formation of the active ferryl species.

## Experimental Section

**General:** All manipulations were carried out by using standard Schlenk techniques. Starting materials were purchased from Acros. Solvents were purchased from Merck and were used without further purification. − UV/Vis: Varian Cary 5E equipped with a temperature controller was used. − MS: Quattro II (Micromass, Manchester, UK) triple quadrupole electrospray mass spectrometer. Typical optimized values for the source parameters were: capillary 2.94 kV, counterelectrode 0.4 kV, source temperature 80 °C, RF lens 0.7 V, skimmer lens offset 5 V, cone voltage 25 V. − EPR: X-band Bruker ER 200 E and 300 spectrometers were used. Low-temperature experiments were performed using an Oxford Instruments continuous-flow liquid-helium cryostat and a temperature-control system. − Syntheses of $L^5$ and $[L^5FeCl]PF_6$: The ligand and the complex were prepared as described in the literature.[3]

**Synthesis of the Complex $[L^5FeCl]Cl$:** A solution of 200 mg (1 mmol) of $FeCl_2 \cdot 4\ H_2O$ in 10 mL of THF was added to a solution of $L^5$ (1 mmol) in 10 mL of THF. A yellow powder precipitated and was collected by filtration (yield 50%). − ESI MS; *m/z*: 438 ($[L^5FeCl]^+$). − $C_{21}H_{25}Cl_2FeN_5 \cdot 2.5\ H_2O$ (519.2): calcd. C 48.58, H 5.82, N 13.49; found C 48.67, H 5.16, N 12.93. − UV/Vis: $\lambda_{max} = 385$ nm ($\varepsilon = 1750\ м^{-1} \cdot cm^{-1}$).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[17] The resonance at $g = 9.3$ is detected only at 4.0 K.
[18] As explained by R. Aasa, T. Vännegard, *J. Magn. Reson.* **1975**, *9*, 308−315, the intensity of the spectrum was multiplied by the temperature. The new spectrum so obtained presents also a peak at $g = 7.5$. The area under this peak decreased with the temperature. Care was taken to avoid saturation conditions.
[19] Abbreviations: bispic$Bz_2$en: *N,N′*-dibenzyl-*N,N′*-bis(2-pyridylmethyl)ethane-1,2-diamine; bispic$Me_2$en: *N,N′*-dimethyl-*N,N′*-bis(2-pyridylmethyl)ethane-1,2-diamine.

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