A Mössbauer Study of $[Fe(edta)(O_2)]^{3-}$ Agrees with a High-Spin Fe^{III} Peroxo Complex

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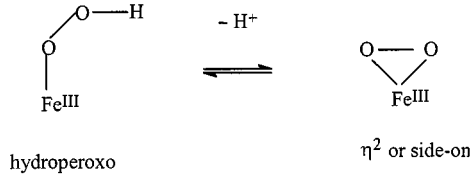
An isotopically enriched $[^{57}Fe(edta)(O_2)]^{3-}$ complex, formed by the addition of H_2O_2 to a $^{57}Fe^{III}$-edta (edta = ethylenediaminetetraacetic acid) complex at elevated pH (pH = 11.3), was studied by Mössbauer spectroscopy. All Mössbauer parameters were determined for this nonheme high-spin ferric peroxo complex with an η^2-FeO_2 (side-on) arrangement by a numerical treatment of applied field Mössbauer data. The peroxo complex exhibits an isomer shift $\delta_{/Fe}$ = 0.65(1) mm s^{-1} and a quadrupole splitting ΔE_Q = +0.72(2) mm s^{-1} (T = 4.2 K). The isomer-shift value is very similar to the value of $\delta_{/Fe}$ = 0.61 mm s^{-1} published for the side-on peroxo complex $[Fe^{III}(N_4Py)(\eta^2\text{-}OO)]^+$ (Py = pyridine) [V. Vrajmasu, E. Münck, R. Ho, L. Que, Jr., G. Roefles, B. L. Feringa, *J. Inorg. Biochem.* **2001**, *86*, 472]. The set of Mössbauer parameters obtained herein is expected to aid the characterization of analogous species that may appear during dioxygen activation by nonheme mononuclear iron enzymes.

Introduction

The mechanism of dioxygen activation by nonheme iron systems has received much attention recently, and it has become clear that peroxo iron intermediates form a central part of these processes.[1] This has been thoroughly documented for diiron enzymes such as ribonucleotide reductase and methane monooxygenase, for which detailed spectroscopic characterizations of peroxo forms have been reported.[1b] Peroxo iron intermediates are also postulated for mononuclear nonheme iron enzymes, although their occurrence has been shown only in the antitumor drug bleomycin whose "activated" form was demonstrated to contain an Fe^{III}-OOH unit.[2] Similar species are likely to be involved in other mononuclear iron enzymes such as Fe-based superoxide dismutase,[3] and superoxide reductase.[4]

Great efforts are currently being made to obtain biologically relevant mononuclear nonheme iron(III) peroxide complexes. Some iron(III) hydrogenperoxo and peroxo complexes, which were proposed or demonstrated to contain a side-on peroxo group, have been identified in solution.[5] In some cases, these two forms can be interchanged by a protonation-deprotonation sequence (Scheme 1):[5a,5c,5d,5f]



Scheme 1

The reactivity of the enzymatic systems will be dictated by the decomposition mode of the peroxide, which obviously depends on its protonation state and coordination geometry. Therefore a detailed investigation of the electronic structure of peroxo-iron species is a prerequisite to understanding their chemistry.

The only nonheme Fe^{III}-peroxo complex studied in any detail is the $[Fe(edta)(O_2)]^{3-}$ complex, a side-on peroxo species.[5g] $[Fe(edta)(O_2)]^{3-}$ was recently characterized by a wide range of spectroscopic techniques, including electron paramagnetic resonance (EPR), low-temperature absorption, variable temperature variable field magnetic circular dichroism (VTVH MCD) and resonance Raman spectroscopy; all the spectroscopic results, together with theoretical calculations, gave insight into the electronic structure of the complex.[6]

Mössbauer spectroscopy has proven invaluable to characterize biological iron sites[7] and it has been instrumental in probing the mechanism of action of the iron-containing

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component of ribonucleotide diphosphate reductase RNR R2,[8] and the hydroxylase component of methane monooxygenase MMOH[9] and stearoyl-ACP Δ^9-desaturase Δ9D.[10] Partial Mössbauer data have recently been published for the side-on peroxo complex $[Fe^{III}(N_4Py)(\eta^2\text{-}OO)]^+$ (Py = pyridine).[11] This prompted us to use Mössbauer spectroscopy to investigate the electronic structure of such complexes and their chemistry with respect to proton exchange. In this paper we report the Mössbauer characterization of the $[Fe(edta)(O_2)]^{3-}$ complex. Our study is a follow up from the work of Solomon et al.,[6] where Mössbauer spectroscopy was not used; we made use of their spectroscopic data [zero-field splitting (ZFS) parameters D and E]. The set of Mössbauer parameters obtained herein is expected to aid the characterization of analogous species that may appear during dioxygen activation by nonheme mononuclear iron enzymes.

Results and Discussion

The complex $[^{57}Fe(edta)(O_2)]^{3-}$ was synthesized following the procedure used by Solomon,[6] by adding H_2O_2 to a $^{57}Fe^{III}$-edta complex at elevated pH (a pH value of 11.3 was used) in buffered solution. It exhibits two weak EPR resonances at $g = 9.9$ and $g = 9.2$, and an intense derivation-type signal in the $g = 3-6$ region. Those resonances were shown to belong to an $S = 5/2$ system with rhombicity $E/D = 0.21$ and $D = -1.0 \pm 0.25$ cm^{-1}.[6,12]

The peroxo complex was studied by Mössbauer spectroscopy at low temperature in a low and a high magnetic field applied parallel to the propagation direction of the Mössbauer γ-rays. Figure 1 shows the Mössbauer spectra of $[Fe(edta)(O_2)]^{3-}$ in a 50 mT magnetic field (T = 4.2 K and 10 K), as well as the contribution of each Kramers doublet.

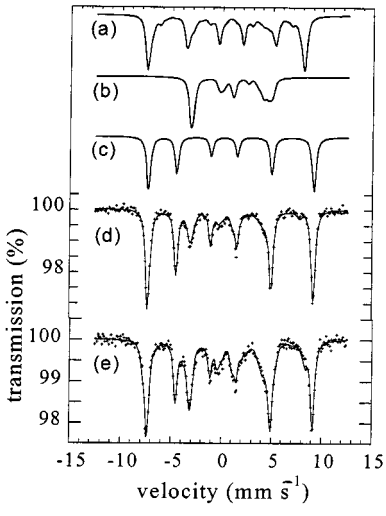

Figure 1. Mössbauer spectra of the $[Fe(edta)(O_2)]^{3-}$ complex; the experimental spectra shown in (d) and (e), taken at T = 4.2 K and T = 10 K, respectively in an applied parallel field of 50 mT, were fitted (solid curves) with the parameter set of Table 1; the solid curves in (a) – (c) show the contribution of each Kramers doublet

The peroxide complex Mössbauer spectrum was also recorded in a 7.0 T magnetic field (T = 4.2 K) and this is shown in Figure 2.

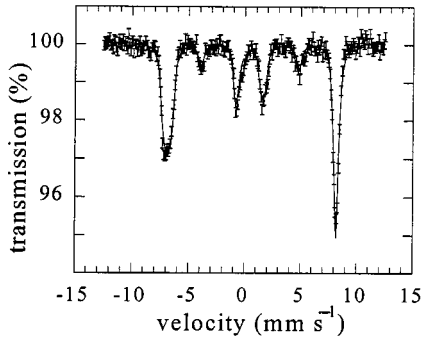

Figure 2. Mössbauer spectrum of the $[Fe(edta)(O_2)]^{3-}$ complex taken at 4.2 K in an applied parallel field of 7.0 T; the solid line corresponds to the fit of the spectrum with the set of Mössbauer parameters from Table 1

These spectra can be simulated within the spin-Hamiltonian formalism (see Exp. Sect. for details). All spectra were simultaneously fitted at the slow-relaxation limit using a unique set of parameters. The ZFS parameters were fixed to the values $D = -1.0$ cm^{-1} and $E/D = 0.21$.[6] The parameters obtained from the fits are shown in Table 1.

Table 1. Spin Hamiltonian parameters for the $[Fe(edta)(O_2)]^{3-}$ complex at T = 4.2 K; the electric field gradient (EFG) tensor, the hyperfine coupling tensor and the zero-field splitting tensor are colinear

	$[Fe(edta)(O_2)]^{3-}$
D (cm^{-1})	-1.0 cm^{-1} [6]
DE/D	0.21[6]
$g_x = g_y = g_z$	2.0
$A_x/g_n\beta_n$ (T)	$-23.1(3)$
$A_y/g_n\beta_n$ (T)	$-21.4(9)$
$A_z/g_n\beta_n$ (T)	$-20.8(1)$
η	0.4(2)
ΔE_Q (mm s^{-1})	+0.72(2)
$\delta_{/Fe}$ (mm s^{-1})	0.65(1)

Varying the ZFS parameter D from -1.25 cm^{-1} to -0.75 cm^{-1} changed the values of the set of parameters in Table 1 very slightly (except E/D, which was fixed at 0.21);[6] however, the best fit was obtained for $D = -1.0$ cm^{-1}.

For $E/D = 0.21$ ($D = -1.0$ cm^{-1}), the lower ("$M_S = \pm 5/2$") Kramers doublet of the $S = 5/2$ species is almost uniaxial and yields a sextuplet spectrum [Figure 1(c)]; the upper Kramers doublet ("$M_S = \pm 1/2$") is not uniaxial but also yields a sextuplet spectrum [Figure 1(a)]. The middle Kramers doublet ("$M_S = \pm 3/2$") is much more magnetically isotropic and yields a more complex-looking spectrum localized between -3.5 and $+6$ mm s^{-1} [Figure 1(b)].[13] At T = 4.2 K, the population of the upper doublet is significally less than that of the ground doublet. Therefore, the

contribution of the upper doublet to the experimental Mössbauer spectrum [Figure 1(d)] is negligible relative to the contribution from the lower and middle doublets. At T = 10 K, the populations of the upper and middle doublets and their contributions to the experimental spectrum increase [Figure 1(e)]. The intensity ratio of the spectral components is related to the Boltzmann factor and thus to the energy differences between the Kramers doublets, which, in turn, are related to the ZFS parameters D and E of the peroxo complex. At 77 K, the Mössbauer spectrum of the peroxo complex (50 mT applied magnetic field, data not shown) was poorly fitted at the slow relaxation limit, and at 200 K a broad unresolved spectrum was obtained (data not shown). This indicates that the fast relaxation limit is still not reached at this temperature.[14]

The $[Fe(edta)(O_2)]^{3-}$ complex exhibits an isomer shift $\delta_{/Fe}$ of 0.65 mm s^{-1} at 4.2 K and a quadrupole splitting $\Delta E_Q = +0.72$ mm s^{-1}. The former value is very similar to the value of 0.61 mm s^{-1} reported for the side-on peroxo complex $[Fe^{III}(N_4Py)(\eta^2\text{-}OO)]^+$.[11] Unfortunately, no quadrupole splitting value is available. Both parameters are also similar to those obtained by Mössbauer spectroscopy for the high-spin Fe^{III}-peroxo porphyrin $[Me_4N][Fe(OEP)O_2$ complex (OEP = octaethylporphyrin dianion);[15] this complex was suggested to contain a side-on peroxo group.[16] The porphyrin peroxo complex exhibits isomer shift and quadrupole splitting values (0.67 mm s^{-1} and + 0.62 mm s^{-1}, respectively, at 4.2 K) similar to those obtained for the $[Fe(edta)(O_2)]^{3-}$ complex, despite different iron environments. In addition, μ-1,2-peroxodiferric complexes exhibit isomer shifts in the range 0.60−0.66 mm s^{-1};[10] the isomer shift of the $[Fe(edta)(O_2)]^{3-}$ complex corresponds to the upper limit of this scale. The hyperfine coupling tensor of the peroxo complex is negative ($A_{av}/g_n\beta_n = -21.8$ T) because of the main contribution from the Fermi contact term (high-spin ferric iron case), and is also slightly anisotropic. For the $[Fe^{III}(N_4Py)(\eta^2\text{-}OO)]^+$ complex, a slighty anisotropic hyperfine tensor was also used with a similar average value ($A_{av}/g_n\beta_n = -21.1$ T).[11] For the $[Me_4N][Fe(OEP)O_2]$ complex, the hyperfine tensor was assumed to be isotropic ($A/g_n\beta_n = -23.3$ T).[14]

The absorbance of the peroxo complex at 520 nm (peroxo-to-iron ligand-to-metal charge transfer transition, LMCT band)[6] was recorded in solution at room temperature as a function of pH; it has been shown that this LMCT band reaches a plateau around pH = 10 (peroxide complex fully formed) and then collapses continuously to vanish at pH = 7.0.[17] In addition, no new bands appear between 400 and 800 nm (data not shown). It is thus not possible to generate, under those conditions, a hydrogenperoxo species by protonation of the peroxo complex, as discussed by Mc Kenzie et al,[5a] Girerd et al,[5c,5d] and Que et al.[5f] with nitrogen ligands.

In conclusion, we have fully characterized a mononuclear nonheme Fe^{III}-peroxo complex, where the peroxo group is coordinated to the iron center in a side-on way, by ^{57}Fe Mössbauer spectroscopy. The set of Mössbauer parameters obtained for the $[Fe(edta)(O_2)]^{3-}$ complex are close to those published for the high-spin ferric porphyrin complex $[Me_4N][Fe(OEP)O_2]$, which was suggested to contain a side-on peroxo group. The isomer shift value for the $[Fe(edta)(O_2)]^{3-}$ complex is also very close to the one reported by Münck et al. for the complex $[Fe^{III}(N_4Py)(\eta^2\text{-}OO)]^+$.[11] Therefore, our Mössbauer study of $[Fe(edta)(O_2)]^{3-}$ agrees with a high-spin Fe^{III} side-on peroxo complex. We are currently characterizing new mononuclear nonheme peroxo complexes by Mössbauer spectroscopy in our laboratory.

Experimental Section

All Mössbauer measurements were performed with a constant acceleration spectrometer calibrated with hematite, and isomeric shifts are reported relative to an Fe metal standard at room temperature. Variable temperature experiments were carried out with the sample placed in the tail section of a variable temperature cryostat. Temperatures of the sample were regulated to within ±0.2 K by a conventional PID system. The sample for Mössbauer spectroscopy contains about 4 mM ^{57}Fe. The Mössbauer spectra were analyzed, in a least-square fit procedure, by using the Hamiltonian **H** describing the system:

$$\mathbf{H} = D[\mathbf{S}_z^2 - (1/3)S(S+1) + (E/D)(\mathbf{S}_x^2 - \mathbf{S}_y^2)] + \beta_e\mathbf{.S.}[g]\mathbf{.B} + <\mathbf{S}>\mathbf{.}[A]\mathbf{.I} - g_n\beta_n\mathbf{B.I} + \mathbf{H_Q}$$

where D and E are the axial and rhombic zero field splitting parameters, respectively, β_e the electronic Bohr magneton, [g] the electronic tensor (considered here to be isotropic with a value fixed to 2), <**S**> the appropriately taken spin expectation value, [A] the magnetic hyperfine tensor, g_n the nuclear factor, β_n the nuclear Bohr magneton and $\mathbf{H_Q}$ the quadrupolar interaction Hamiltonian.[18] Each spectrum was then fitted with the set of parameters obtained when all the spectra were simultaneously fitted; the discrepancies found from this initial set allowed us to estimate the errors in each parameter.

Synthesis of the complex $[^{57}Fe(edta)(O_2)]^{3-}$

The $[^{57}Fe(edta)(O_2)]^{3-}$ complex was synthesized as reported recently.[8] Basically, a CHES (Sigma) buffered solution of fivefold excess ethylenediaminetetraacetic acid, tetrasodium salt (edta, Sigma) at pH = 11.3 was allowed to react with solid $^{57}FeCl_3{\cdot}6H_2O$. This mixture was heated until total dissolution of the solid material. The Fe^{III}-edta complex concentration was about 4 mM. The pH of the solution was then readjusted to 11.3 with sodium hydroxide and then filtered. The purple peroxo complex $[^{57}Fe(edta)(O_2)]^{3-}$ was formed by addition of a 10-fold excess of 30% H_2O_2 (Aldrich) to the former solution. For isotopic enrichment, the $^{57}FeCl_3{\cdot}6H_2O$ starting compound was obtained by dissolving $^{57}Fe_2O_3$ (AMT Ltd.) in 20 equivalents of concentrated HCl (Carlo Erba) under reflux and then by evaporating to dryness.

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