Catalase vs Peroxidase Activity of a Manganese(II) Compound: Identification of a Mn(III)\(-\mu-O)_{2}\)Mn(IV) Reaction Intermediate by Electrospray Ionization Mass Spectrometry and Electron Paramagnetic Resonance Spectroscopy

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Herein, we report reactivity studies of the mononuclear water-soluble complex [Mn(II)(HPClNOL)(\eta_{1}-NO_{3})(\eta_{2}-NO_{3})]\_1, where HPClNOL = 1-(bis-pyridin-2-ylmethyl-amino)-3-chloropropan-2-ol, toward peroxides (H\_2O\_2 and tert-butylhydroperoxide). Both the catalase (in aqueous solution) and peroxidase (in CH\_3CN) activities of 1 were evaluated using a range of techniques including electronic absorption spectroscopy, volumetry (kinetic studies), pH monitoring during H\_2O\_2 disproportionation, electron paramagnetic resonance (EPR), electrospray ionization mass spectrometry in the positive ion mode [ESI(+)-MS], and gas chromatography (GC). Electrochemical studies showed that 1 can be oxidized to Mn(III) and Mn(IV). The catalase-like activity of 1 was evaluated with and without pH control. The results show that the pH decreases when the reaction is performed in unbuffered media. Furthermore, the activity of 1 is greater in buffered than in unbuffered media, demonstrating that pH influences the activity of 1 toward H\_2O\_2.

For the reaction of 1 with H\_2O\_2, EPR and ESI(+)-MS have led to the identification of the intermediate [Mn(III)Mn(IV)(\mu-O)\_2(PClNOL)\_2]\+. The peroxidase activity of 1 was also evaluated by monitoring cyclohexane oxidation, using H\_2O\_2 or tert-butylhydroperoxide as the terminal oxidants. Low yields (<7%) were obtained for H\_2O\_2, probably because it competes with 1 for the catalase-like activity. In contrast, using tert-butylhydroperoxide, up to 29% of cyclohexane conversion was obtained. A mechanistic model for the catalase activity of 1 that incorporates the observed lag phase in O\_2 production, the pH variation, and the formation of a Mn(III)-(\mu-O)\_2-Mn(IV) intermediate is proposed.

Introduction

Oxygen, though essential for aerobic metabolism, can be converted to toxic metabolites, such as the superoxide anion, hydroxyl radical, and hydrogen peroxide, collectively known as reactive oxygen species (ROS). These ROS are associated with numerous pathological conditions including ischemic and inflammatory processes.\(^1\) All living cells therefore use exquisitely orchestrated mechanisms to control the level of ROS, including the use of endogenous antioxidant metal-
loenzymes such as the superoxide dismutases (SODs) and catalases (CATs), which catalyze the dismutation of superoxide and the disproportionation of $\text{H}_2\text{O}_2$, respectively. Furthermore, $\text{H}_2\text{O}_2$ is employed by heme-containing peroxidases to oxidize various substrates, including organic compounds (phenol), proteins (cytochrome c), peptides (glutathione), NADPH, fatty acids. Recently, they have been employed to oxidize dyes and other pollutant compounds. CATs and peroxidases are widespread and protect against the build-up of dangerous concentrations of hydrogen peroxide in living systems, which is formed as a consequence of only partial reduction of dioxygen. However, if the production of $\text{H}_2\text{O}_2$ exceeds the capacity of these enzymes, $\text{H}_2\text{O}_2$ becomes a substrate for the Fenton reaction, forming the extremely toxic and mutagenic hydroxyl radicals (OH$^\cdot$).

While the majority of CATs rely on the heme group for catalysis, an alternative class of manganese-dependent CATs (MnCATs) has been identified in three bacterial organisms: Lactobacillus plantarum, Thermus thermophilus, and Thermoleophilum album. Recent crystallographic studies revealed an elegant structure in which the active sites of the Mn(II,III) and Mn(III,IV) oxidation states can also be determined, their catalytic mechanism is still a topic of discussion. Further, H$_2$O$_2$ is employed by heme-containing peroxidases to oxidize various substrates, including organic compounds (phenol), proteins (cytochrome c), peptides (glutathione), NADPH, fatty acids. Recently, they have been employed to oxidize dyes and other pollutant compounds. CATs and peroxidases are widespread and protect against the build-up of dangerous concentrations of hydrogen peroxide in living systems, which is formed as a consequence of only partial reduction of dioxygen. However, if the production of $\text{H}_2\text{O}_2$ exceeds the capacity of these enzymes, $\text{H}_2\text{O}_2$ becomes a substrate for the Fenton reaction, forming the extremely toxic and mutagenic hydroxyl radicals (OH$^\cdot$).

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in aqueous solutions. The HPCINOL ligand was employed successfully in the synthesis of copper and iron complexes, which are functional models for nucleases.25 Here, the HPCINOL ligand was employed to synthesize the mononuclear water-soluble complex \([\text{Mn}(II)(\text{HPCINOL})(\eta^1-\text{NO}_3)(\eta^2-\text{NO}_2)])\), 1 (Scheme 1). Characterization of 1 by X-ray diffraction and ESI(+)-MS/MS has been published.26 In this work, we have evaluated the catalytic ability of MnCATs. The reaction was monitored by following O2 evolution, ESI(+)-MS, EPR, electronic absorption spectroscopy, and following changes in pH. The studies were performed with and without pH control, employing TRIS/TRIS·HCl (TRIS: tris(hydroxymethyl)aminomethane) as buffer. Since the activation of peroxide molecules by metal compounds is of interest for promoting the oxidation of organic molecules (peroxidase activity), we also present data concerning the catalytic ability of 1 in promoting the oxidation of cyclohexane using \(\text{H}_2\text{O}_2\) and tert-butylhydroperoxide (t-BuOOH) as oxidants.

### Experimental Section

**Materials.** Dimethylformamide (DMF) was distilled in vacuum and stored under an argon atmosphere prior to electrochemical studies. All the other reagents and solvents were of analytical and/or spectroscopic grade and were used without further purification.

**Ligand and Complex Syntheses.** The ligand HPCINOL was synthesized by a reaction between the secondary amine bmpa and epichlorohydrin, as reported previously.27 The mononuclear \([\text{Mn}(II)-(\text{HPCINOL})(\eta^1-\text{NO}_3)(\eta^2-\text{NO}_2)])\) complex 1 was prepared according to a published procedure (Scheme 1).26

**Spectroscopic Methods.** UV−vis spectra were recorded in water on a Shimadzu 1601 PC UV−vis spectrophotometer. EPR spectra were recorded on a Bruker Elexsys E580 EPR spectrometer equipped with a Bruker ER036TM Teslameter and frequency counter for calibration of the magnetic field and microwave frequency, respectively. Low temperature (140 K) at the sample position employed a nitrogen flow-through system in conjunction with a liquid nitrogen Eurotherm ER4131vt temperature controller. Experimental conditions were as follows: peroxide titrations were performed in buffer solutions (TRIS/TRIS·HCl, 1.0 mol·L\(^{-1}\); pH 7.2). and samples were rapidly frozen in liquid nitrogen at specified time intervals. Each experiment was conducted by adding 8 \(\mu\)L of a hydrogen peroxide solution (stock concentration: 0.55 and 4.4 mol·L\(^{-1}\), respectively) to 180 \(\mu\)L of a 1 mmol·L\(^{-1}\) solution of the complex 1. Computer simulation of the dimanganese EPR spectra employed Molecular Sophe28 in conjunction with Octave to allow the optimization of the spin Hamiltonian parameters.

**ESI(+)-MS.** The experiments were performed using a Q-TOF mass spectrometer (Micromass, Manchester, UK). The ionization technique used was electrospray ionization in the positive ion mode (ESI(+)-MS). Typical conditions were the following: source and desolvation temperatures of 353 K, and capillary and cone voltages of 3 kV and 40 V, respectively. To a solution of 1 (1 mL, 1 × 10\(^{-6}\) mol·L\(^{-1}\), MeOH:\(\text{H}_2\text{O}\) 1:1) was added 50 \(\mu\)L of \(\text{H}_2\text{O}_2\) (23%) and the reaction was followed over 20 min. The sample was injected using a syringe pump (Harvard Apparatus) at a flow rate of 10 \(\mu\)L·min\(^{-1}\). Mass spectra were acquired over the 50−1500 m/z range.

**Electrochemistry.** Electrochemical studies were performed with an Autolab PGSTAT 10 potentiostat/galvanostat in acetonitrile containing 0.1 M tetrabutylammonium perchlorate (TBAClO\(_4\)) as the supporting electrolyte under an argon atmosphere at room temperature. The electrochemical cell employed a standard three-electrode configuration: a glassy carbon working electrode, a platinum-wire auxiliary electrode, and a commercial Ag/AgCl electrode immersed in a salt bridge containing 0.1 M TBAClO\(_4\). The ferrocene/ferroenium (Fc/Fc\(^+\)) couple (0.400 V vs NHE) was used as an internal standard.29

**Catalase-like Activity.** All the reactions between 1 and \(\text{H}_2\text{O}_2\) were performed in buffered (TRIS/TRIS·HCl, 0.1 mol·L\(^{-1}\); pH 7.2, \(\text{LiClO}_4\) 0.1 mol·L\(^{-1}\)) and in unbuffered water solutions (\(\text{LiClO}_4\) 0.1 mol·L\(^{-1}\)). Initially, the reactivity of 1 toward \(\text{H}_2\text{O}_2\) was investigated in water (\(\text{LiClO}_4\) 0.1 mol·L\(^{-1}\)) via UV−vis spectroscopy. A solution of 1 was prepared and was kept at room temperature for 12 h prior to use. Then, 10 \(\mu\)L of \(\text{H}_2\text{O}_2\) (21%) was added to the aqueous solution of the complex (5 × 10\(^{-3}\) mol·L\(^{-1}\), 3 mL), and spectra were recorded at 90 s intervals in a 1 cm path length cell. A similar study was performed using a buffered system (TRIS/TRIS·HCl, 0.1 mol·L\(^{-1}\); pH 7.2, \(\text{LiClO}_4\) 0.1 mol·L\(^{-1}\)).

Volumetric measurements of the evolved dioxygen produced during the reactions of 1 with \(\text{H}_2\text{O}_2\) were performed in triplicate as follows: a 10 mL round-bottom flask containing an aqueous solution of 1 (1 × 10\(^{-3}\) mol·L\(^{-1}\), 5.00 mL) and \(\text{LiClO}_4\) (0.1 mol·L\(^{-1}\), 1 mL) was placed in a water bath (298.00 ± 0.01 K). The flask was closed by a rubber septum and a cannula was used to connect the reaction flask to an inverted graduated pipet, filled with water. While the solution containing the complex was being stirred, a solution of \(\text{H}_2\text{O}_2\) diluted to appropriate concentrations was injected through the rubber septum using a microsyringe. The volume of oxygen produced was measured in the pipet. Experiments were performed keeping the concentration of the substrate constant and varying the concentration of the complex to determine the order with respect to the complex. A similar study was performed to obtain the order with respect to the substrate.

The pH variation during \(\text{H}_2\text{O}_2\) disproportionation was carried out in triplicate under unbuffered conditions as follows: a flask containing an aqueous solution of the complex (1 × 10\(^{-3}\) mol·L\(^{-1}\), 5.00 mL) and \(\text{LiClO}_4\) (0.1 mol·L\(^{-1}\), 1 mL) was placed in a water bath (298.00 ± 0.01 K). The pH was determined by using a Micronal model B374 pH meter fitted with an Analion model V631 electrode. A solution of \(\text{H}_2\text{O}_2\) diluted to appropriate concentration with water was then added to this solution, which was stirred and the pH values were measured at 5 s time intervals.


(29) Octave v 3.0.1 can be obtained from www.octave.org.

Peroxidase-like Activity. The reactions were performed in a 50 cm³ round-bottom flask under stirring for 24 h. The catalyst: substrate:oxidant ratio was 1:1000:1000 with the following reagent amounts: 0.75 cm³ of cyclohexane (7 × 10⁻³ mol), 0.59 cm³ of H₂O₂ or 0.93 cm³ of t-BuOOH (7 × 10⁻³ mol), and 3.3 mg of I (7 × 10⁻⁶ mol). Acetonitrile (MeCN) and t-butanol (t-BuOH) were used as solvent (10 cm³), and the experiments were performed at two temperatures: room temperature (rt) and 50 °C. The reactions were quenched by adding an aqueous 0.4 mol·L⁻¹ solution of Na₂SO₄, followed by extraction with 10 cm³ of diethyl ether. The ether layer was dried with anhydrous Na₂SO₄ and analyzed by GC. The aqueous phase was titrated with NaOH to quantify the total of acid compounds obtained in the reaction expressed as adipic acid.

Results and Discussion

Electrochemical Properties of 1. Results obtained by cyclic and pulse differential voltammetry in DMF (Figure S1, Supporting Information) indicate the presence of two redox processes at 0.65 V (ΔEₚ = 56 mV) and 0.92 V (ΔEₚ = 40 mV) vs Fc/Fc⁺. The processes are attributed to the oxidation of the Mn(II) center to Mn(III) and then to Mn(IV), respectively. These values are shifted to more positive potential with respect to the corresponding redox couple presented by compound [Mn(III)(bpia)Cl₂](ClO₄), which presents processes at 0.61 V and E₁/₂ = 1.53 V vs SCE.² seventeen

Catalase-like Activity of 1 Measured by UV–vis Spectroscopy. When H₂O₂ is added to a colorless aqueous solution of 1, it becomes greenish-brown and liberates O₂. The progress of the reaction between 1 and H₂O₂ was monitored in water by UV–vis spectroscopy (Figure 1). Complex 1 shows no absorption features in the visible range, and its unique band in the UV range (λ = 255 nm, ε = 1.2 × 10⁴ L·mol⁻¹·cm⁻¹) is due to a π → π* transition involving the pyridine groups of the ligand.³¹ This feature is similar to that exhibited by the MnCAT from T. thermophilus in the (II,II) oxidation state, which also lacks features in the visible absorption spectrum.³² Immediately after the addition of 10 µL of H₂O₂ (6.85 mol·L⁻¹) to the aqueous solution (3 mL) of 1 (1 × 10⁻³ mol·L⁻¹), poorly resolved absorption bands or shoulders near 410, 539, and 620 nm are observed (absorbance = 0.35, 0.11, and 0.083, respectively).³³ Similar absorption bands (three absorption bands at 400–700 nm) were observed when the dimanganese(II,II) complexes [Mn₂(L)(OAc)₂(CH₃OH)](ClO₄) and [Mn₂(L)(OBz)(H₂O)](ClO₄), reported by Latour, were allowed to react with H₂O₂ in acetonitrile. Mn(III)Mn(IV)-di-µ-oxo complexes containing the tripodal ligands bpia,⁴ seventeen tpa, bpg, and pda also exhibit similar absorption bands. Using the assignment proposed by Solomon et al.,⁴ the band at highest energy has been assigned to an oxo → Mn(IV) charge-transfer transition (LMCT), while the others (λ = 500–560 nm, λ = 590–600 nm) are related to Mn(IV) d–d transitions. Krebs has proposed that the highest energy band also has a d–d contribution while the lowest energy band is related to a LMCT oxo → Mn(IV).¹⁷ Thus, the presence of similar absorption bands in 1, upon the addition of H₂O₂, suggests that a [Mn(III)−(µ-O)₂−Mn(IV)] intermediate is formed.

![Figure 1. UV–vis spectra of 1 (dotted line) after the addition of H₂O₂: [I] = 1 × 10⁻³ mol·L⁻¹; 3 mL, [H₂O₂] = 6.18 mol·L⁻¹, 10 mL, temperature = 298 K. The spectra were recorded after 90 s incubation. Water was used as solvent. (top) Unbuffered system. (bottom) Buffered system. Insets show the increase and decrease in absorption of the bands at 410 (■), 539 (●), and 630 nm (▲) (buffered) after baseline correction.³³](image-url)
The reaction between 1 and H₂O₂ was also performed in aqueous solution at constant pH, using a TRIS/TRIS·HCl buffer (pH 7.2; Figure 2). Similar absorption features were observed but now are about 3–5-fold more intense than in the unbuffered system (i.e., 410, 541, and 630 nm, absorbance = 1.74, 0.43, and 0.30, respectively). After some time the intensity of the absorption bands decreased in the unbuffered solution but remains more stable in the buffered system (insets in Figure 1-bottom). It is therefore possible that in the unbuffered environment the active species [Mn(III)–(µ-O)₂–Mn(IV)] is formed and consumed during the catalytic cycle. Similar behavior was observed for the complex Mn(II)edda reported by Rush and Maskos. Mn(II)edda also forms a Mn(III)–(µ-O)₂–Mn(IV) species in the presence of H₂O₂ and the drop in intensity of the absorption bands was attributed to the reaction between Mn(II) and Mn(III)Mn(IV), resulting in Mn(III) species. A similar process may occur with 1 or the degradation of the Mn(IV)–(µ-O)₂–Mn(III) core may result from the presence of H⁺ ions in solution (vide infra).

Catalase-like Activity and Kinetics of H₂O₂ Disproportionation. The H₂O₂ disproportionation promoted by 1 was investigated in water, at 298 K, by measuring O₂ evolution. The water solubility exhibited by 1 is of relevance since it enables us to study the catalytic reaction under conditions similar to those encountered by MnCATs in a biological environment. Qualitatively, when H₂O₂ is added to an aqueous solution of 1, the colorless solution turns greenish-brown as O₂ is produced. Gradually, the color changes to yellow and the formation of O₂ decreases. It is worth noting that the catalyst is not able to disproportionate all the H₂O₂ added, which may indicate that the catalytic species is not stable during the process.

The time course of dioxygen evolution is presented in Figures 2 and 3. Owing to the differences in the intensity in the absorption bands observed in the UV–vis spectra for the disproportionation of H₂O₂ promoted by 1 in buffered (pH = 7.2) and unbuffered media (vide supra), kinetic experiments were also performed with and without pH control. To determine the rate law of the reaction, the initial rate method was applied. Here, pseudo-first-order kinetic measurements (see the Supporting Information) in unbuffered and buffered solutions indicate that the rate laws are of the form $k_{obs}[\text{complex}]^{1.5}[\text{H}_2\text{O}_2]^{0.7}$ and $k_{obs}[\text{complex}]^{1.1}[\text{H}_2\text{O}_2]^{1.3}$, respectively. Although it is not possible to compare the rates measured under these two different conditions directly, it is evident that in the buffered system oxygen is produced in larger quantity (about 4–6-fold) and more rapidly (Figures 2 and 3). The deviations from first-order dependence of the rate on the concentrations of substrate and complex may be due to the presence of several species of 1 in solution (i.e., mononuclear, dinuclear, and trinuclear). Furthermore, an inspection of the progress curves in Figures 2 and 3 reveal the presence of a small lag phase, which indicates that the active species is formed after H₂O₂ addition.

For several dinuclear manganese catalase biomimetics, a first order dependence of the reaction rate with respect to substrate and catalyst concentration has been observed. Latour and co-workers employed tripodal ligands (tpa, H(bpg), H₂(pda), and H₃(nta)) in the preparation of a set of dinuclear di-µ-oxo manganese (Mn(III)Mn(IV) and Mn(I-II)Mn(III)) compounds with catalase activity. Their results indicated that the disproportionation activity is favored by a lower oxidation state (Mn(III)Mn(III) > Mn(III)Mn(IV)) and by increasing the number of carboxylate groups on the ligand structure (pda > bpg > tpa). They suggested that the higher oxidation state of Mn(III) favors the disproportionation reaction, while the presence of more carboxylate groups allows for a more stable complex formation.

activity of compounds containing carboxylate rich ligands might be assigned to the ability of carboxylate groups to act as an internal base, which may indicate that H\(^+\) ions were also released in the reaction of these compounds with H\(_2\)O\(_2\). A study of the catalase activity for the compound Mn(edda) revealed that it is inactive below pH 5.15, suggesting that the presence of H\(^+\) ions affect its reactivity. Thus the higher activity presented by I in buffered solutions maybe due to the prevention of H\(^+\) inactivation.

**Catalase-like Activity and pH Variation.** Since it was observed in both electronic absorption and kinetic experiments that the reactivity of I is dependent on the pH of the medium, the variation of the pH during H\(_2\)O\(_2\) decomposition was monitored.

In the unbuffered system (Figure 4), three distinct steps of pH variation are observed. An initial rapid drop in pH is followed by a transient and rapid increase in pH. Subsequently, a slow exponential pH decrease is recorded. The time related to the first change in pH can be directly correlated to the time of the lag phase observed in the kinetic measurements (Figures 2 and 3), indicating that, during the formation of the active species, protons are released. In the second phase of the pH profile, it is possible to suggest that the protonation of the \(\mu\)-oxo group leads to a transient increase in pH, while in the third phase the gradual conversion of H\(_2\)O\(_2\) to O\(_2\) results in the release of protons. O\(_2\) production stops when the pH reaches 4.5. Since a higher catalytic efficiency is observed (Figures 2 and 3) for I when the disproportionation reaction is performed in a buffered system, (pH = 7.2), it is likely that this is a result of the absence of protonation-induced inactivation.

For the reaction between Mn(II)(edda) and H\(_2\)O\(_2\), Rush reported a similar variation of pH to that observed for I (Figure 4).\(^{35}\) However, in the Mn(II)(edda) system, the pH turns back returns to the initial values, while for I it decreases exponentially.

**Interaction of 1 and H\(_2\)O\(_2\) by ESI(+)MS.** The gentleness and broadness of ESI and the ability of ESI-MS to provide proper and continuous snapshots of solution constituents have been used extensively by us and others to probe reaction mechanisms involving organic and inorganic species.\(^{36}\) The ESI(+)-MS spectrum for complex I has been previously reported.\(^{26}\) In a H\(_2\)O:MeOH solution (1:1), I is at equilibrium with at least four cationic species, which indicates that the single mononuclear solid-state structure of I is not totally preserved in solution, since tri-, di-, and mononuclear complexes with compositions [Mn(II)\(_3\)-(HPClNOL)\(_3\)(NO\(_3\))\(_3\)]\(^{1+}\), [Mn(II)(PCiNOL)\(_2\)(NO\(_3\))\(_3\)]\(^{2+}\), [Mn(II)-(HPClNOL)\(_2\)2(NO\(_3\))\(_3\)]\(^{3+}\), and [Mn(II)(HPClNOL)\(_2\)2(NO\(_3\))\(_3\)]\(^{4+}\) of 4348, 931, 878, and 699, respectively, were transferred from solution to gas phase and characterized by ESI(+)MS.\(^{26}\) Complex I is neutral and could not be detected as such by ESI(−)-MS.

To probe the mechanism of H\(_2\)O\(_2\) disproportionation promoted by I, the reaction was monitored by ESI(-)-MS at room temperature (Figure 5). An appropriate volume of H\(_2\)O\(_2\) was added and the resulting reaction solution directly infused into the ESI source of a Q-TOF mass spectrometer. Spectra were recorded before and after (0.2, 3.0, 9.0, and 20 min) the addition of H\(_2\)O\(_2\) (Figure 5a−e). Immediately after the addition of H\(_2\)O\(_2\), a new ion of m/z 722 emerges and its abundance increases as a function of reaction time. After 20 min (Figure 5e), the ion of m/z 722 is still abundant, indicating its substantial stability under these experimental conditions. This species may be assigned as the monocation [(HPClNOL)Mn(III)-(\(\mu\)-oxo)\(_2\)-Mn(IV)]\(^{2+}\)/[(HPClNOL)Mn(II)-(\(\mu\)-per-

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toward H$_2$O$_2$ was also investigated by measuring changes in higher concentrations ([H$_2$O$_2$]$_{\text{final}}$ 9 min; and (e) 20 min. Insets display the calculated (f) and experimental (g) isotopic patterns for the ion of m/ɛ 722, that is: [Mn(III)(PCINOL)-(µ-oxo)-Mn(IV)(PCINOL)]$^+$ = Mn$_2$C$_6$H$_{12}$N$_6$O$_4$Cl$_2$.

**Figure 5.** ESI(+)-MS for the reaction in water at 25 °C between I and H$_2$O$_2$: (a) before H$_2$O$_2$ addition; (b) after 0.2 min of H$_2$O$_2$ addition; (c) 3 min; (d) 9 min; and (e) 20 min. Insets display the calculated (f) and experimental (g) isotopic patterns for the ion of m/ɛ 722, that is: [Mn(III)(PCINOL)-(µ-oxo)-Mn(IV)(PCINOL)]$^+$ = Mn$_2$C$_6$H$_{12}$N$_6$O$_4$Cl$_2$.

Interaction of 1 with H$_2$O$_2$ by EPR. The reactivity of 1 toward H$_2$O$_2$ was also investigated by measuring changes in the EPR spectra at two different H$_2$O$_2$ concentrations. The EPR spectrum of 1 ([H$_2$O$_2$]$_{\text{final}}$: 9.57 × 10$^{-4}$ mol·L$^{-1}$) in TRIS/HCl (pH 7.2) buffer solution at 140 K is shown in Figure 6a. This spectrum has broad features at low field, $g_{\text{eff}}$ ∼ 5 and $g_{\text{eff}}$ ∼ 3, and a broad feature at $g_{\text{eff}}$ = 2 that are characteristic of a dinuclear Mn(II)Mn(II) species described by the groups of Pecoraro$^{17}$ and Dubois.$^{18,21}$ After the addition of H$_2$O$_2$ at a low concentration ([H$_2$O$_2$]$_{\text{final}}$ = 2.34 × 10$^{-3}$ mol·L$^{-1}$; Figure 6b) to 1 the spectrum changes with the appearance of a typical 16-line signal centered around $g$ = 2 that is attributed to a Mn(IV)Mn(III) species.$^{17,18,21}$ With the addition of H$_2$O$_2$ at higher concentrations ([H$_2$O$_2$]$_{\text{final}}$ = 1.87 × 10$^{-1}$ mol·L$^{-1}$; Figure 6c), the 16-line spectrum increases in intensity and the Mn(II)Mn(II) spectrum disappears. After the addition of a second aliquot of H$_2$O$_2$ ([H$_2$O$_2$]$_{\text{final}}$ = 3.74 × 10$^{-1}$ mol·L$^{-1}$), the spectrum changes with the partial loss of the 16-line signal and the appearance of a new signal at $g$ = 2 with a six-line Mn hyperfine structure ($A_{\text{eff}}g_{\beta}$ ∼ 8.9 mT), characteristic of an isolated mononuclear Mn(II) species (Figure 6d). With the addition of a third aliquot of H$_2$O$_2$ ([H$_2$O$_2$]$_{\text{final}}$ = 5.61 × 10$^{-1}$ mol·L$^{-1}$; Figure 6e), the 16-line spectrum was completely absent and the only species seen by EPR is mononuclear Mn(II). At each point in the titration, the sample was thawed and incubated at 45 °C for several minutes but no further changes to the EPR spectra were observed.

The spin Hamiltonian for a dinuclear heterovalent Mn(III)-Mn(IV) center (eq 1) incorporates electron Zeeman, fine structure and Mn hyperfine coupling terms for each ion and the isotropic and anistropic exchange coupling.

$$H = -2J_{\text{iso}}S_1S_2 + S_1JS_2 + \sum_{j=1}^{2} \beta BgS_j + S_{\alpha j}B_{\text{Mn}(j)}$$

where $S_{\alpha j}$ is the Mn magnetic moment, $B_{\text{Mn}(j)}$ is the Mn magnetic field, and $\beta$ is the Bohr magneton.

Since the antiferromagnetic exchange coupling in a di-µ-oxo bridged species is extremely large ($|J| > 100$ cm$^{-1}$) with $\beta g_{\alpha j} > |J|$, the spin states ($S_T$) can be described by $S_T = |S_1 + S_2|, |S_1 + S_2| - 1,..., |S_1 - S_2|$ (i.e., $S_T = 1/2, 3/2, 5/2, 7/2$) in the strong exchange regime. Consequently at low temperatures only the $S_T = 1/2$ spin state will be populated. Computer simulation of the 16-line EPR spectrum (Figures 6c and 7a) was performed with the following spin Hamiltonian for an $S = 1/2$ coupled representation:

$$H = \beta BgS + SA_{\text{Mn}(\text{II})} + S_{\alpha j}B_{\text{Mn}(j)}$$

having the following $g$ and $A$(Mn) matrices: $g_x = 2.0014, g_y = 2.0030, g_z = 1.9865; A(\text{Mn}(\text{III})) = 136.3 \times 10^{-4}$

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reactivity of 1. Kinetic, spectroscopic and spectrometric data presented herein and mechanistic models described previously, a scheme for the reaction mechanism employed by 1 is proposed (Figure 8). Importantly, at room temperature, there is no evidence for the involvement of free radicals. The proposed model accounts for the experimentally observed species, including the formation of a Mn(III)–μ-O–Mn(IV) intermediate, oxygen evolution, and the observed pH changes in unbuffered reactions.

In the initial phase, there is an equilibrium between the mono-, di-, and trinuclear forms of 1, with the dimeric form being predominant in buffered solutions as reflected in the EPR spectra (Figure 6). Reaction of 2 molecules of 1 (Mn(II)–Mn(II)) and two molecules of H₂O₂ produces two molecules of complex A with a Mn(III)–μ-O–Mn(III) core. Subsequently, in the presence of H₂O₂, a “dimer-of-dimers” species (complex B) is formed with a di-μ-oxo group as a bridge between the two dimanganese complexes. Concomitant with the formation of this interdimer di-μ-oxo bridge is the oxidation of Mn(III) to Mn(IV). Thus, the first step in the formation of this tetramer should be the formation of an interdimer peroxide bridge, as proposed previously by Okawa. A similar tetranuclear manganese compound containing three di-μ-oxo units was published previously. The proposed release of protons associated with the formation of species A and B contributes to the initial drop in pH observed in the unbuffered reactions (Figure 4). The decomposition of complex B results in the formation of the mixed-valence complex with a Mn(III)–μ-O–Mn(IV) core (complex C) and release of H₂O. The experimental (ESR and ESI(+)-MS) observation of this mixed valence species indicates that this is an intermediate in the reaction mechanism and, further, that the rate determining step (protonation of species C to yield species D) occurs after its formation. The very low pKₐ of the doubly Lewis activated μ-oxo bridge and the observation that the time

![Figure 6](image6.png)

Figure 6. X-band EPR spectra of 1 at 140 K in a buffered solution, ν = 9.34854 GHz: (a) prior to the addition of H₂O₂, (b) frozen immediately after the addition of 1.8% H₂O₂, (c) frozen immediately after the addition of 8 μL of 15% H₂O₂, (d) a second aliquot (8 μL) of 15% H₂O₂ was added to sample c and frozen after 2 min incubation, (e) a third aliquot (8 μL) of 15% H₂O₂ (H₂O₂final = 5.61 × 10⁻¹ M) was added to sample d and frozen after 2 min incubation. 

![Figure 7](image7.png)

Figure 7. X-band (ν = 9.34854 GHz) EPR spectrum of 1 at 140 K in a buffered solution (pH 7.2): (a) experimental spectrum of complex 1 frozen immediately after the addition of 8 μL of 15% H₂O₂ (H₂O₂final = 1.87 × 10⁻¹ M), (b) computer simulation; see text for details.

Mechanistic Implications for the Catalase-like Reactivity of 1. On the basis of the kinetic, spectroscopic and spectrometric data presented herein and mechanistic models described previously, a scheme for the reaction mechanism employed by 1 is proposed (Figure 8). Importantly, at room temperature, there is no evidence for the involvement of free radicals. The proposed model accounts for the experimentally observed species, including the formation of a Mn(III)–μ-O–Mn(IV) interme-

span from mixing 1 and peroxide to the formation of species C accounts exactly for the observed lag in O₂ evolution (Figures 2 and 3) are also consistent with the rate determining step being the protonation of the µ-oxo bridge in species C. Thus, accumulation of species C and protons leads to a burst of proton consumption with a concomitant rise in pH (Figure 4) and formation of species D. Subsequently, complex D reacts with H₂O₂ (µ-η¹:η¹ coordination) forming complex E, which releases O₂, generating complex F. The coordination of a further H₂O₂ molecule to complex F results in the release of H⁺ ions and formation of species G. This species undergoes H₂O elimination with concomitant two-electron oxidation of the Mn(II)Mn(III) core (complex G), leading to the formation of the experimentally observed [(PCINOL)Mn(III)µ-O]₂Mn(IV)(PCINOL)]⁺ (complex C) intermediate again completing the catalytic cycle (species C–G). Importantly, there is a net release of protons in the catalytic cycle which leads to the gradual decrease of pH over the time course of the reaction (Figure 4). The EPR studies revealed that a mononuclear Mn(II) species is obtained after incubation with excess of H₂O₂ (Figure 6d and e). This observation is interpreted in terms of an inactivation of the catalytic complex F by protonation of the oxo bridge or ligand molecule (below pH 4.5, the complex is inactive). The gradual inactivation of complex F results in decreased dioxygen evolution (Figures 2 and 3), decreased amounts of species C (Figure 6), and incomplete consumption of hydrogen peroxide, all of which are observed experimentally. Similar inactivation at low pH has been observed previously, and consequently, the proposed reaction mechanism should be considered pseudo-catalytic.

as a special example of peroxidases in which the substrate is catalyzed by catalases. These enzymes may be regarded

yield;

the oxidation of particular organic compounds using H2O2 characterized as heme containing proteins that catalyze

Scheme 2.


In contrast, typical peroxidases use H2O2 to perform oxygenations of various substrates.3 Thus, peroxidases have been corroborated with other manganese compounds.46–48 For the manganese complex to act as an oxidation catalyst, it is required that the oxidized state of the catalyst oxidizes the organic substrate instead of oxidizing H2O2.46

We have thus investigated whether 1 may also utilize peroxide (H2O2, t-BuOOH) molecules in an alternative reaction pathway, namely the oxidation of cyclohexane.

Cyclohexanol (Cy—OH), cyclohexanone (Cy=O), cyclohexyl hydroperoxide (Cy—OOH), tert-butyl cyclohexyl peroxide (Cy—OOtBu), and adipic acid (AA) were formed (Table 1) during the oxidation process (Scheme 2). The greatest yield was obtained when t-BuOOH was used as an oxidant at 50 °C (experiments 4 and 8 in Table 1). The effect of solvent (acetone or t-BuOH) was negligible. With H2O2 as oxidant, very low product yields were obtained (experiments 1, 2, 5, and 6 in Table 1). Thus, whereas the reaction between 1 and H2O2 is bifunctional, the disproportionation reaction is preferred. In contrast, t-BuOOH appears to be a suitable substrate for performing oxygenation reactions with 1. Recently, we have analyzed the oxidation of cyclohexane with H2O2 and t-BuOOH as oxidant, using a family of mononuclear iron(III) compounds with similar ligands (i.e. [Fe(PABMPA)Cl2], [Fe(MPBMPA)Cl2], [Fe(PBMPA)Cl2], and [Fe(PABMPA)Cl3]).49,50 In contrast to 1, cyclohexane oxidation in the presence of these iron compounds is more efficient when H2O2 is employed as the oxidant. Especially for [Fe(PABMPA)Cl2], for which we have also characterized their catalase activity,50 the yield obtained in the cyclohexane oxidation with H2O2 was 33.4% as compared to a marginal 5.4% yield when t-BuOOH was employed. For these iron compounds, Cy—OOH and Cy—OOr-Bu were identified as products of the cyclohexane oxidation, suggesting the operation of a Fenton or Haber–Weiss mechanism.51,52

The lack of cyclohexyl peroxides as products in the reactions between 1 and H2O2 at room temperature indicates that no radical species are formed under these conditions. The identification of Cy—OOH at 50 °C shows, however, that a side reaction other than H2O2 disproportionation is taking place under this condition. When t-BuOOH was used as an oxidant, a larger amount of alkyl peroxide was identified as the product with no O2 production, suggesting that the interaction between t-BuOOH and 1 forms radical species.

**Table 1. Results for the Cyclohexane Oxidation Catalyzed by 1 after 24 h<sup>a</sup>**

<table>
<thead>
<tr>
<th>entry</th>
<th>T (°C)</th>
<th>oxidant</th>
<th>solvent</th>
<th>CyOH</th>
<th>Cy=O</th>
<th>Cy—OOH</th>
<th>Cy—OOr-Bu</th>
<th>AA</th>
<th>total</th>
<th>CyOH/Cy—O</th>
<th>TN&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rt</td>
<td>H2O2</td>
<td>MeCN</td>
<td>1.3</td>
<td>1.0</td>
<td>3.8</td>
<td>0.13</td>
<td>0.13</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>H2O2</td>
<td>MeCN</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.14</td>
<td>0.63</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>rt</td>
<td>t-BuOOH</td>
<td>MeCN</td>
<td>8.9</td>
<td>15</td>
<td>0.21</td>
<td>4.4</td>
<td>0.16</td>
<td>28.7</td>
<td>0.59</td>
<td>286.7</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>t-BuOOH</td>
<td>MeCN</td>
<td>0.49</td>
<td>0.35</td>
<td>1.0</td>
<td>0.16</td>
<td>2</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>rt</td>
<td>H2O2</td>
<td>t-BuOH</td>
<td>0.35</td>
<td>0.74</td>
<td>1.2</td>
<td>0.29</td>
<td>0.13</td>
<td>3.8</td>
<td>0.85</td>
<td>26.2</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>H2O2</td>
<td>t-BuOH</td>
<td>1</td>
<td>13.7</td>
<td>0.8</td>
<td>3.8</td>
<td>0.85</td>
<td>26.2</td>
<td>0.52</td>
<td>262.5</td>
</tr>
<tr>
<td>7</td>
<td>rt</td>
<td>t-BuOOH</td>
<td>t-BuOH</td>
<td>7.1</td>
<td>13.7</td>
<td>0.8</td>
<td>3.8</td>
<td>0.85</td>
<td>26.2</td>
<td>0.52</td>
<td>262.5</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>t-BuOOH</td>
<td>t-BuOH</td>
<td>8.9</td>
<td>15</td>
<td>0.21</td>
<td>4.4</td>
<td>0.16</td>
<td>28.7</td>
<td>0.59</td>
<td>286.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio catalyst:substrate:oxidant = 1:1000:1000. <sup>b</sup> R<sub>c</sub> = (R<sub>c</sub> × n<sub>i</sub>)<sup>c</sup>/n<sub>c</sub>; R<sub>c</sub> = R<sub>AA</sub> + (R<sub>c</sub> × n<sub>i</sub>)<sup>c</sup>; R<sub>c</sub> = corrected chromatographic yield; R<sub>c</sub> = chromatographic yield; n<sub>c</sub> = n<sub>i</sub> − n<sub>s</sub>; R<sub>s</sub> = adipic acid yield. * TN = turnover number; calculated as moles of products per mole of catalyst.

**Scheme 2. Products of the Cyclohexane Oxidation Performed by 1**

Oxidation of Cyclohexane by 1. H2O2 disproportionation is catalyzed by catalases. These enzymes may be regarded as a special example of peroxidases in which the substrate oxidized by H2O2 is another molecule of H2O2.45 Conversely, typical peroxidases use H2O2 to perform oxygenations of various substrates.3 Thus, peroxidases have been characterized as heme containing proteins that catalyze the oxidation of particular organic compounds using H2O2 as an electron acceptor. Catalase and peroxidase activities have been corroborated with other manganese compounds.46–48 For the manganese complex to act as an oxidation catalyst, it is required that the oxidized state of the catalyst oxidizes the organic substrate instead of oxidizing H2O2.46


Supporting Information Available: Differential pulse voltammogram and kinetic plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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