

A Functional Model of Manganese Catalase. Mass Spectrometric and Visible Spectral Evidence for $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$ and $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(\text{=O})$ Intermediates

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A dinuclear manganese(II) complex $[\text{Mn}_2(\text{L})(\text{PhCO}_2)_2(\text{NCS})]$ [$\text{L} = 2,6\text{-bis}\{N\text{-}[2\text{-(dimethylamino)ethyl]iminomethyl}\}\text{-}4\text{-methylphenolate}(1^-)$] decomposes H_2O_2 catalytically in dimethylformamide solution; two oxomanganese(IV) species $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$ and $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(\text{=O})$ are detected for the first time as intermediates in the H_2O_2 disproportionation reaction based on mass spectrometric and visible spectral studies.

Manganese catalases (Mn-CAT) have recently been found in three different origins: *Lactobacillus plantarum*,¹ *Thermus thermophilus*² and *Thermoleophilum album*.³ For the first two Mn-CATs, the presence of a pair of Mn ions at the active site has been shown based on X-ray structure analysis,⁴ electron paramagnetic resonance (EPR)⁵ and extended X-ray absorption fine structure (EXAFS)⁶ studies. The $\mu\text{-oxo-bis}(\mu\text{-car}$

boxylato)dimanganese(III) core structure has been suggested⁷ based on visible spectral characteristics, but the detailed structure of the active site is still unknown. Some functional models have been reported to date⁸⁻¹² but the active species in each H_2O_2 disproportionation reaction were poorly characterized, with the exception of a few cases.⁹ Here we report direct evidence for the presence of $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$ and

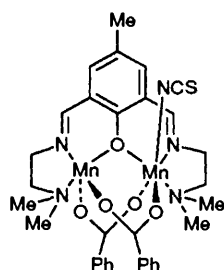


Fig. 1 Chemical structure of $[\text{Mn}_2(\text{L})(\text{PhCO}_2)_2(\text{NCS})]$

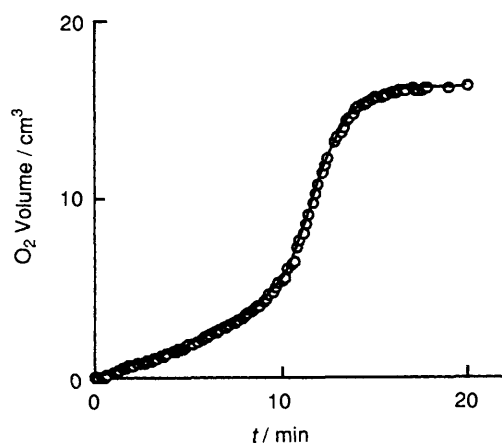


Fig. 2 Time course of O_2 -evolution in H_2O_2 disproportionation by **1**. Conditions: **1** ($5 \mu\text{mol}$) in DMF (2 cm^3), H_2O_2 (9.9%, 0.5 cm^3 ; 1.45 mmol), at 0°C

$\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(\text{=O})$ intermediates in the H_2O_2 disproportionation reaction using a μ -phenoxo-bis(μ -carboxylato)dimanganese(II) complex.

A dinuclear manganese(II) complex $[\text{Mn}_2(\text{L})(\text{PhCO}_2)_2(\text{NCS})]$ [$\text{L} = 2,6\text{-bis}\{N\text{-}[2\text{-(dimethylamino)ethyl]imino-methyl}\}\text{-4-methylphenolate}(1^-)$] (Fig. 1) has been synthesized and its μ -phenoxo-bis(μ -carboxylato)dimanganese(II) core structure has been proved based on X-ray structural analysis.¹³ The complex behaves as a 1:1 electrolyte in dimethylformamide (DMF),¹³ and fast atom bombardment (FAB) mass spectral studies indicate that the complex dissociates into $[\text{Mn}_2(\text{L})(\text{PhCO}_2)_2]^+$ **1** and NCS^- ion. When H_2O_2 was added to a DMF solution of the complex, catalytic decomposition of H_2O_2 occurred with more than 1000 turnovers based on volumetric measurements of evolved dioxygen (Fig. 2). The initial rate was slow, but after a lag period the rate significantly increased and the colour of the solution changed from yellow to purple. The purple solution showed an intense absorption band ($\epsilon \sim 2000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) around 530 nm onto which fine structures were imposed, separated by $\sim 730 \text{ cm}^{-1}$ (Fig. 3). The fine structure may be assigned to the $\nu(\text{Mn}=\text{O})$ vibration¹⁴ coupled to a ligand-to-metal charge transfer (LMCT) (from O^{2-} to Mn) band¹⁵ through vibronic interaction.

The purple solution was submitted to FAB mass spectrometry and new significant ions at m/z 671 (**2**) and 687 (**3**) (Fig. 4) were detected, which correspond to the compositions (**1** + O) and (**1** + 2O), respectively, from the exact mass measurements under high resolution conditions. The ions **2** and **3** were shifted to m/z 673 and 691, respectively, when $\text{H}_2^{18}\text{O}_2$ was added. The increments of one O atom in **2** and two O atoms in **3** evidently originate from H_2O_2 . The collision-activated dissociation (CAD) experiments of **2** and **3** (Fig. 5) have revealed that **2** does not originate from **3**. Further, the very similar dissociation pattern in CAD of **2** and **3** suggests the same bonding mode of the O^{2-} ion in both species. μ -Oxo- and di- μ -oxodimanganese complexes are commonly known,⁷

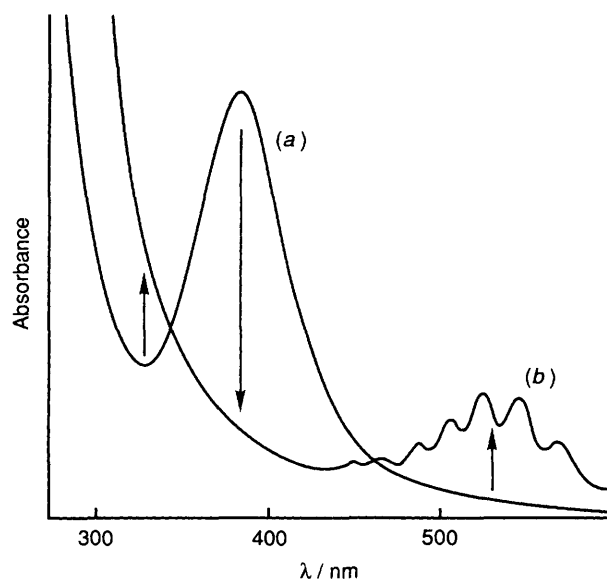


Fig. 3 Visible spectral changes on adding H_2O_2 (10.0%, 0.5 cm^3) to a DMF solution (2 cm^3) of **1** ($0.2 \mu\text{mol}$): (a) just after the addition of H_2O_2 , (b) after 30 min

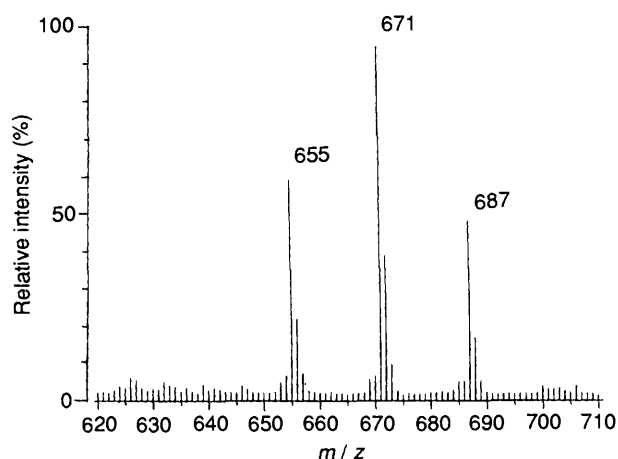


Fig. 4 Positive ion FAB mass spectrum of an aqueous DMF solution of **1** and H_2O_2 with *m*-nitrobenzyl alcohol (NBA) matrix. Conditions: **1** (ca. $0.07 \mu\text{mol}$) in DMF (ca. 0.05 cm^3), H_2O_2 (10%, ca. 0.05 cm^3 ; ca. $40 \mu\text{mol}$), after ca. 30 min

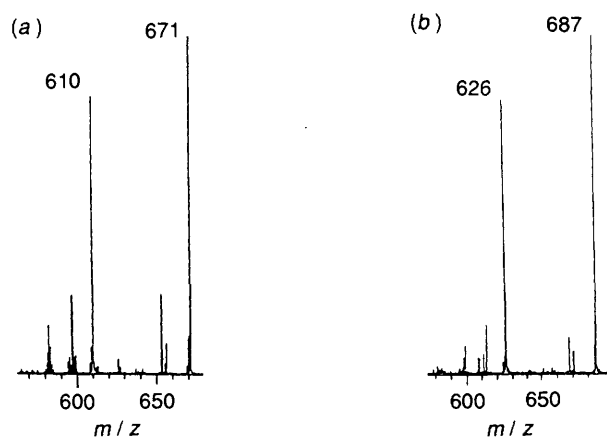
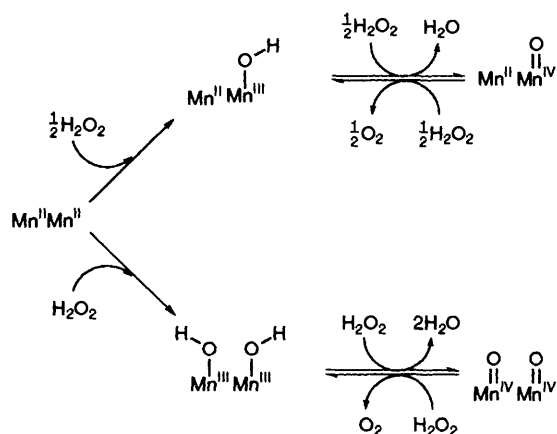


Fig. 5 Positive ion CAD spectra of **2** (a) and **3** (b) generated by FABMS



Scheme 1 Proposed mechanism of the H_2O_2 disproportionation reaction of **1**

but the bridging function of O^{2-} in **2** and **3** is ruled out because μ -oxo- μ -phenoxo-bis(μ -carboxylato)- and di- μ -oxo- μ -phenoxobis(μ -carboxylato)dimanganese core structures are unknown. From these results together with visible spectral and mass spectrometric results the only possible bonding mode of the oxygen is $\text{Mn}^{\text{IV}}=\text{O}$, and **2** and **3** can be formulated as $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(\text{=O})$ and $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$, respectively, retaining the original μ -phenoxo-bis(μ -carboxylato)dimanganese core structure.

We have also noticed, based on time-dependence of FAB mass spectra, that **2** is formed at an early stage of the H_2O_2 disproportionation reaction whereas **3** appears after the lag period. The initial complex between **1** and H_2O_2 must be $[\text{Mn}_2(\text{L})(\text{PhCO}_2)_2(\text{OOH})]$, which is converted into the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{OH})$ species through the peroxo-bridged dimeric intermediate $\{\text{Mn}_2(\text{L})(\text{PhCO}_2)_2\}_2(\text{O}_2^{2-})$ or to the $\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ species through the μ -peroxo-intermediate $\{\text{Mn}_2(\mu\text{-O}_2^{2-})(\text{L})(\text{PhCO}_2)_2\}$. Further oxidation of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{OH})$ and $\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ species with H_2O_2 forms **2** and **3**, respectively. The lag period before the formation of **3** can be explained by the fact that the μ -peroxo intermediate is hardly formed owing to the steric requirement of **L** rendering five-coordinate geometry about one metal ion.¹³

Based on the above discussion a mechanism for the H_2O_2 disproportionation reaction of **1** is proposed (Scheme 1). The $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$ species **3** oxidises H_2O_2 to O_2 producing the $\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ species which in turn reduces H_2O_2 to water reproducing the $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$ species. Both $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2$ and $\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ inevitably have *cis* configuration[†] with respect to the two oxo or hydroxo groups so that the cycle between the two species is easily performed by the 'chelating' interaction

[†] This is based on the inspection of the molecular structure of $[\text{Mn}_2(\text{L})(\text{MeCO}_2)_2(\text{NCS})]$ (ref. 13) and the formation mechanism of $[\text{Mn}^{\text{III}}(\text{OH})_2]$ discussed in the text.

with H_2O_2 , in accord with a high catalytic activity of **3**. On the other hand, the cycle between $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(\text{=O})$ and $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{OH})$ must be slower since this involves intermolecular 'bridging' interaction with H_2O_2 . This leads to a low catalytic activity of **2**.

In this study the $\text{Mn}^{\text{IV}}=\text{O}$ species were detected as intermediates in H_2O_2 disproportionation reaction for the first time. We presume that the cycle $\{\text{Mn}^{\text{IV}}(\text{=O})\}_2-\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ is relevant to biological Mn-CAT. Further details of this study will be reported elsewhere together with studies using related complexes such as $[\text{Mn}_2(\text{L}^1)(\text{MeCO}_2)_2(\text{NCS})]^{16}$ [$\text{L}^1 = 2,6$ -bis(*N*-(2-pyridylethyl)iminomethyl)-4-methylphenolate(1-)] and $[\text{Mn}_2(\text{L}^2)(\text{PhCO}_2)_2](\text{ClO}_4)^{17}$ [$\text{L}^2 = 2,6$ -bis(bis(2-pyridylmethyl)aminomethyl)-4-methylphenolate(1-)].

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