

Dinuclear Mn complexes as functional models of Mn catalase

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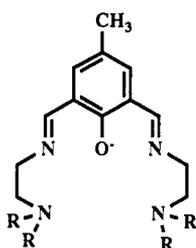
Abstract: μ -Phenoxo-bis(μ -carboxylato)dimanganese complexes with a core structure of C_s symmetry show a high catalytic activity to disproportionate H_2O_2 ($2H_2O_2 \rightarrow 2H_2O + O_2$), whereas complexes with a core structure of C_2 symmetry and mononuclear Mn complexes show only a low activity. For the C_s symmetric complexes, a dinuclear cis - $\{Mn^{IV}(=O)\}_2$ intermediate has been detected in the H_2O_2 disproportionation reaction and a mechanistic scheme to catalyze the H_2O_2 disproportionation through the interconversion cis - $\{Mn^{IV}(=O)\}_2/cis$ - $\{Mn^{III}(OH)\}_2$ is proposed. It is shown that the equivalence of two Mn ions in electronic nature is essential to show a CAT-like activity.

A. INTRODUCTION

Hydrogen peroxide is extremely toxic to living cells. However it can be disproportionated ($2H_2O_2 \rightarrow 2H_2O + O_2$) by non-heme manganese catalases (MnCATs). For the MnCATs from *Lactobacillus plantarum* (1) and *Thermus thermophilus* (2), the involvement of a pair of Mn ions at the active site has been proved by various studies. In particular a Mn--Mn separation of ca. 3.6(3) Å is reported for MnCAT from *T. thermophilus* based on X-ray crystallography (3), but the active site structure and the catalytic mechanism of MnCAT remain unclear. It is suggested from visible spectroscopic studies that the μ -oxo-bis(μ -carboxylato)dimanganese(III) core is a promising candidate for the active site of MnCAT (4). Some dimanganese complexes having this core structure are known but these complexes cannot be used for functional model studies because of their instability in solution, especially in the presence of H_2O_2 . We report here a bioinorganic approach to MnCAT using dinucleating ligands that provide stable dinuclear manganese complexes with structures relating to the μ -oxo-bis(μ -carboxylato)dimanganese core.

B. μ -PHENOXY-BIS(μ -CARBOXYLATO)DIMANGANESE CORE COMPLEXES AS FUNCTIONAL MnCAT MODELS

Recently we have reported that the dinucleating ligands 2,6-bis[2-(dialkylamino)ethyliminomethyl]-4-methylphenolate(1-) (alkyl = methyl (L^1) or ethyl (L^2)) form dinuclear manganese(II) complexes $[\text{Mn}_2(\text{L})(\text{RCOO})_2(\text{NCS})]$ ($\text{L}=\text{L}^1$ or L^2 , $\text{R}=\text{Me}$ or Ph) having a μ -phenoxy-bis(μ -carboxylato)dimanganese core (5). These complexes exist as $[\text{Mn}_2(\text{L})(\text{RCOO})_2]^+$ in DMF and catalyze the disproportionation of H_2O_2 with more than 1000 turnovers (Conditions: 5 μmol of a complex and 1.45 mmol of H_2O_2 (9.9 %, 0.5 cm^3) in DMF (2 cm^3) at 0 $^\circ\text{C}$) (6). The time course of O_2 evolution is sigmoidal with a slow initial process and then a fast process appearing after a lag period (Fig. 1). The initial yellow color of the solution changed to deep purple when the fast process occurred. The purple solution showed an intense absorption band around 530 nm ($\epsilon \sim 2000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) on which fine structures, separated by $\sim 730 \text{ cm}^{-1}$, were imposed (Fig. 2). The fine structure can be assigned to the $\nu(\text{Mn}=\text{O})$ mode coupled to a LMCT band (from O^{2-} to Mn) through vibronic interaction.



$(L^1)^- : \text{R}=\text{CH}_3$
 $(L^2)^- : \text{R}=\text{C}_2\text{H}_5$

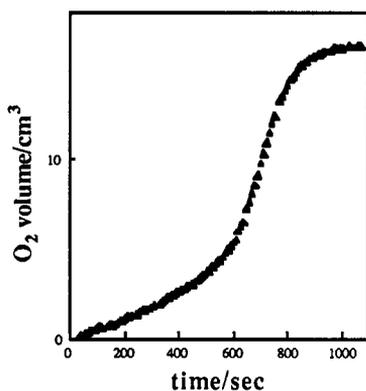


Fig. 1 Catalytic H_2O_2 disproportionation $[\text{Mn}_2(\text{L}^1)(\text{PhCOO})_2(\text{NCS})]$

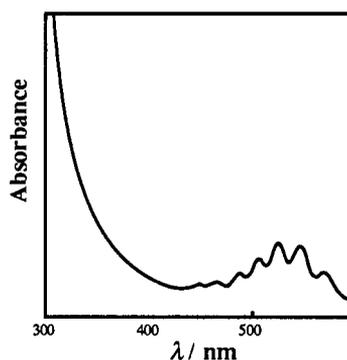


Fig. 2 LMCT band with $\nu(\text{Mn}=\text{O})$ vibration mode

The purple solution containing $[\text{Mn}_2(\text{L}^1)(\text{PhCOO})_2]^+$ (A) was submitted for FAB mass spectrometry and two prominent ions were detected at m/z 671 (B) and 687 (C), which correspond to (A + O) and (A + 2O), respectively. These ions were shifted to m/z 673 and 691, respectively, when $\text{H}_2^{18}\text{O}_2$ was used as the substrate.

Evidently, the oxygen(s) in **B** and **C** originates from the added H_2O_2 . The collision-activated dissociation (CAD) experiments for **B** and **C** have revealed that **B** does not arise from **C**. Further, the very similar dissociation pattern in CAD between **B** and **C** indicates the same bonding mode of the O^{2-} ion in both species. The bridging function of O^{2-} in **B** and **C** is ruled out because μ -oxo- μ -phenoxo-bis(μ -carboxylato)- and di- μ -oxo- μ -phenoxo-bis(μ -carboxylato)dimanganese core structures are unknown. Using the visible spectral and FAB mass spectrometric results discussed above we deduce that the only possible bonding mode of the O^{2-} is $\text{Mn}=\text{O}$. Thus, **B** and **C** can be formulated as $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(=\text{O})$ and $\{\text{Mn}^{\text{IV}}(=\text{O})\}_2$, respectively.

Based on the time-dependence of the FAB mass spectra, **B** is formed at an early stage of the H_2O_2 disproportionation and involved in the slow catalytic reaction whereas **C** appears after the lag period and is involved in a fast catalytic reaction. The initial product between $[\text{Mn}_2(\text{L})(\text{RCOO})_2]^+$ and H_2O_2 must be $[\text{Mn}_2(\text{L})(\text{RCOO})_2(\text{OOH})]$ which is formed by replacing the NCS^- of $[\text{Mn}_2(\text{L})(\text{RCOO})_2(\text{NCS})]$ by OOH^- . The hydroperoxo adduct may be converted into the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{OH})$ species through the intermolecularly peroxo-bridged intermediate $\{\text{Mn}_2\}(\mu\text{-O}_2^{2-})\{\text{Mn}_2\}$ or to the $\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ species through the intramolecularly peroxo-bridged intermediate $\{\text{Mn}(\mu\text{-O}_2^{2-})\text{Mn}\}$. Further oxidation of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{OH})$ species with H_2O_2 forms **B** by an intermolecular mechanism, and oxidation of the $\{\text{Mn}^{\text{III}}(\text{OH})\}_2$ species with H_2O_2 forms **C** by an intramolecular mechanism. Based on the above discussion a mechanistic scheme for the fast catalytic H_2O_2 disproportionation by **C** is proposed (Fig. 3). Such an interconversion between oxo and hydroxo species with prototropy is known in oxygenation-deoxygenation processes at the diiron core in haemerythrin (7).

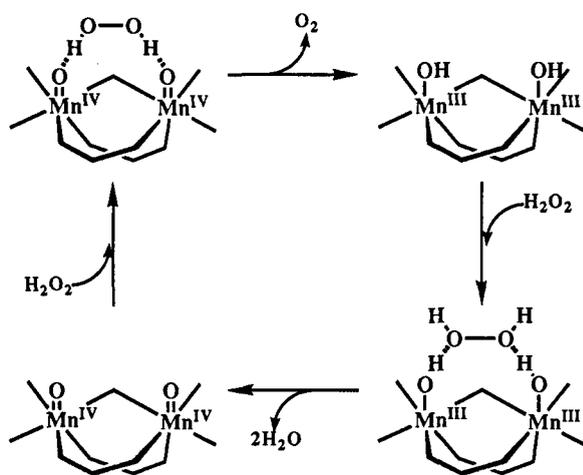
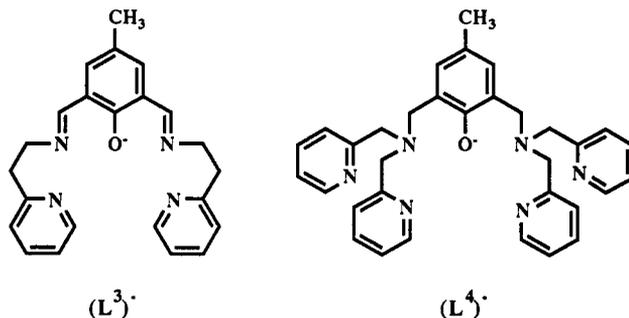


Fig. 3 Mechanistic scheme of catalytic H_2O_2 disproportionation

It is generally believed that two oxidation states, $Mn_2(II,II)$ and $Mn_2(III,III)$, are involved in the catalytic disproportionation of H_2O_2 by MnCAT (8), but this has not yet been proven. The present study implies that the higher oxidation state $Mn_2(IV,IV)$ can be involved in the MnCAT reaction.

C. GEOMETRICAL SIGNIFICANCE OF THE Mn_2 CORE

In the above catalytic decomposition of H_2O_2 , both $\{Mn^{IV}(=O)\}_2$ and $\{Mn^{III}(OH)\}_2$ must have *cis*-configurations with respect to the two oxo or hydroxo groups so as to allow the cycle between the two species by intramolecular mechanism (Fig. 3). The significance of the μ -phenoxo-bis(μ -carboxylato)dimanganese core structure in H_2O_2 disproportionation has been studied for three complexes: $[Mn_2(L^1)(MeCOO)_2(NCS)]$ (1), $[Mn_2(L^3)(MeCOO)_2(NCS)]$ (2) ($L^3=2,6$ -bis[N-(2-pyridylethyl)iminomethyl]-4-methylphenolate (1-) and $[Mn_2(L^4)(PhCOO)_2]ClO_4$ (3) ($L^4=2,6$ -bis[bis(2-pyridylmethyl)aminomethyl]-4-methylphenolate (1-) (9).



The CAT-like function of 1 is depicted in Fig. 1. The complex 2 showed essentially the slow catalytic process; the fast catalytic process barely appeared after a long lag period for this complex. The complex 3 showed only the slow catalytic process.

The space-filling drawing of $[Mn_2(L^1)(MeCOO)_2]^+$, based on the crystal structure, is shown in Fig. 4. The molecular symmetry of the cation is approximated as pseudo C_s with the vacant sites of the two Mn ions in *cis* positions (top view). The other side of the core is perfectly screened by the two carboxylate groups from any attack by hydrogen peroxide (bottom view). Thus, this complex can incorporate H_2O_2 over the Mn_2 core to provide the *cis*- $\{Mn^{III}(OH)\}_2$ and *cis*- $\{Mn^{IV}(=O)\}_2$ species.

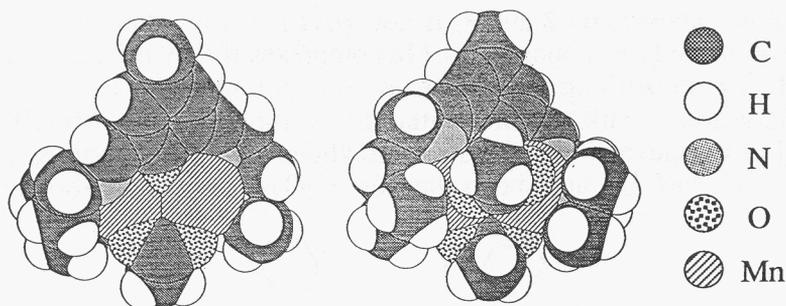


Fig. 4 Space-filling drawing of $[\text{Mn}_2(\text{L}^1)(\text{MeCOO})_2]^+$: top view (left) and bottom view(right)

The cation $[\text{Mn}_2(\text{L}^3)(\text{MeCOO})_2]^+$ has a core structure of C_2 symmetry where the sixth vacant sites of the two manganese ions are *trans* to each other (10) (Fig. 5). The deformation of the *trans* structure (C_2 symmetry) to a *cis* structure (C_s symmetry) is essential for this complex to form the active species, and this seems to be difficult as gauged by the long lag period.

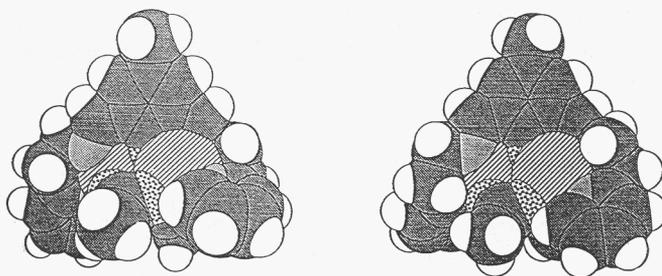
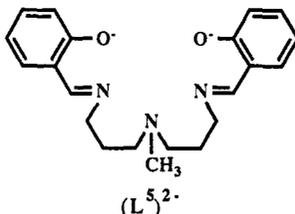


Fig. 5 Space-filling drawing of $[\text{Mn}_2(\text{L}^3)(\text{MeCOO})_2]^+$: top view (left) and bottom view(right)

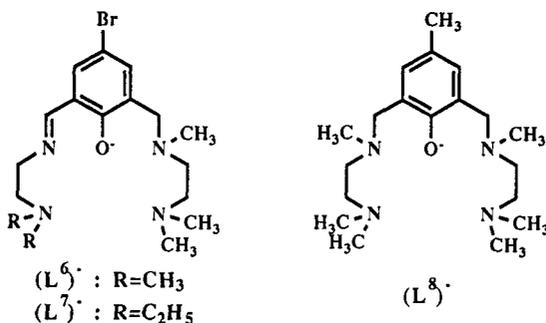
The structure of **3** has not been solved but the crystal structure of the corresponding $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complex $[\text{Mn}_2(\text{L}^4)(\text{PhCOO})_2](\text{ClO}_4)_2$ has been determined (11). The geometry around each Mn is six-coordinate but one of the Mn-N(pyridyl) bonds at each site is elongated and so this site can be made available for H_2O_2 coordination. The labile coordination sites within the Mn_2 core are *trans* to each other, providing pseudo C_2 symmetry to this core. However the deformation of the *trans* structure to a *cis* structure is impossible because of the steric requirement of L^4 ; this is in accord with the slow catalytic function of **3**.

The slow catalysis by **2** and **3** is not specific for the dinuclear structure and may also be observed for mononuclear Mn complexes if they have a vacant or labile site for H_2O_2 coordination and a redox potential necessary for H_2O_2 disproportionation. This is illustrated by a mononuclear Mn(III) complex $[\text{Mn}(\text{L}^5)\text{Cl}]$ of pentadentate N,N' -bis[3-(salicylideneamino)propyl]methylamine(2-) which exists as $[\text{Mn}(\text{L}^4)]^+$ and shows the slow H_2O_2 disproportionation reaction.



D. SIGNIFICANCE OF AN ELECTRONICALLY EQUIVALENT PAIR OF Mn IONS

In order to see the effect of equivalence and non-equivalence of the pair of Mn ions upon CAT-like activity, 4-bromo-2-[[2-(dimethylaminoethyl)methylamino]methyl]-6-[2-(dialkylamino)ethyliminomethyl]phenol (alkyl=methyl (L^6) or ethyl (L^7)) and 2,6-bis[[N-(2-dimethylaminoethyl)-N-methyl]aminomethyl]-4-methylphenolate(1-) (L^8) were prepared. L^6 and L^7 are unsymmetrical with respect to the articular amino and imino nitrogens. L^8 is symmetrical with respect to the two articular nitrogens.



The dinuclear manganese(II) complexes $[\text{Mn}_2(\text{L})(\text{RCOO})_2(\text{NCS})]$ ($\text{L}=\text{L}^6$ or L^7 ; $\text{R}=\text{Me}$ or Ph) have cores with C_s symmetry very similar to that of $[\text{Mn}_2(\text{L}^1)(\text{PhCOO})_2]^+$ (**12**). They resemble the complexes of L^1 and L^2 in their CAT-like activity, showing sigmoidal behavior in the H_2O_2 disproportionation reaction, the $\nu(\text{Mn}=\text{O})$ mode (ca. 730 cm^{-1}) on the LMCT band at 530 nm, and FAB mass spectrometric evidences for the $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}(=\text{O})$ and $\{\text{Mn}^{\text{IV}}(=\text{O})\}_2$ species. One noticeable difference in CAT-like activity is seen in the total amount of evolved dioxygen. That is, the dioxygen evolved was only 60-70 % of the theoretical

amount for the complexes of L⁶ and L⁷ whereas it was 100 % for the complexes of L¹ and L². It should be emphasized that in the reaction with the complexes of L⁶ and L⁷ no intact H₂O₂ remained when the evolution of dioxygen had ceased. On the other hand, the evolution of the theoretical amount of O₂ was observed when [Mn₂(L⁸)(RCOO)₂(NCS)] was used as the catalyst.

Thus, a side reaction occurred to consume H₂O₂ when the Mn₂ complexes of unsymmetrical ligands L⁶ and L⁷ were adopted. At this stage we can only point out that the complexes of L⁶ and L⁷ differ from those of L¹, L² and L⁸ in their electrochemical property, stabilizing the mixed-valent Mn₂(II,III) state. It appears that a heterolytic fission of HO-OH bond occurs when a pair of Mn ions are not equivalent in electronic nature.

Further evidence for the significance of an electronically equivalent Mn pair in MnCAT-like activity has been obtained using the dinuclear Mn(II) complexes [Mn₂(R^{m,n})(AcO)₂] of the phenol-based macrocycles (R^{m,n})²⁻. The macrocycles are symmetrical (m=n=3) or unsymmetrical (m=2 and n=3; m=2 and n=4) with respect to the lateral chains. Heterodinuclear [NiMn(R^{2,3})](ClO₄)₂ and [CuMn(R^{2,3})](ClO₄)₂ have also been prepared for comparison.

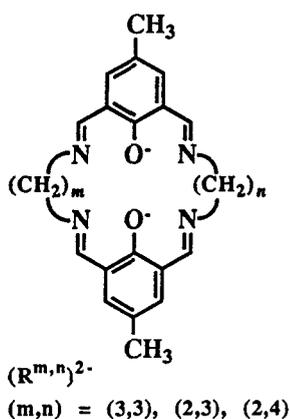


TABLE Amounts of evolved O₂.

Complex	O ₂ (%)
[Mn ₂ (R ^{3,3})(AcO) ₂]	100
[Mn ₂ (R ^{2,3})(AcO) ₂]	~50
[Mn ₂ (R ^{2,4})(AcO) ₂]	~50
[NiMn(R ^{2,3})](ClO ₄) ₂	0
[CuMn(R ^{2,3})](ClO ₄) ₂	0

The reactivities of the Mn₂, CuMn and NiMn complexes toward H₂O₂ were studied under the same conditions and the results are given in Table 1. The heterodinuclear complexes showed no catalytic activity whereas the dinuclear Mn₂ complexes showed an activity. The dioxygen evolution was quantitative for [Mn₂(R^{3,3})(OAc)₂] but it was less than the theoretical amount for [Mn₂(R^{2,3})(OAc)₂] and [Mn₂(R^{2,4})(OAc)₂]. This information lends support to the statement that an electronically equivalent pair of Mn ions is essential for CAT-like activity.

E. CONCLUSION

From the present studies using μ-phenoxo-bis(μ-carboxylato)dimanganese(II) complexes, the prerequisites for a dinuclear Mn₂ core to show a high CAT-like activity have been deduced: (1) that a pair of Mn ions are at an appropriate distance

apart, (2) each Mn has a vacant (or labile) site and the two vacant sites of the Mn₂ core are *cis* to each other in order to allow the incorporation of H₂O₂ at the core, and (3) the pair of Mn ions must be equivalent in electronic nature in order to cause the homolytic fission of HO-OH bond. The active species involved in this CAT-like reaction are presumably *cis*-{Mn^{III}(OH)}₂ and *cis*-{Mn^{IV}(=O)}₂, which are interconverted by the redox reaction with H₂O₂ via an intramolecular mechanism.

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