Synthesis of Di-μ-oxo Manganese(III/IV) Complexes as Functional Models of *L. Plantarum* Pseudo-catalase: Influence of Electronic and Steric Factors; Catalysis and Kinetics of Hydrogen Peroxide Disproportionation

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Di-μ-oxo dimanganese(III/IV) complexes are efficient catalysts for H_2O_2 disproportionation in neutral aqueous medium; the activity can be correlated to the electron density on the metal and the accessibility of the catalytic site.

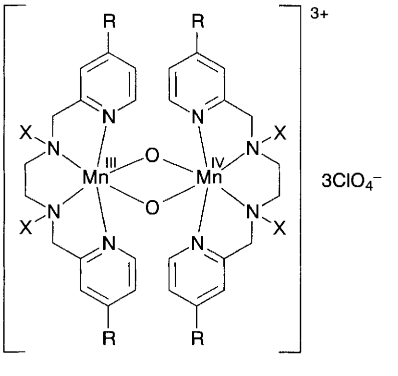
Many multinuclear manganese clusters that are involved in biological systems have been recognised and studied. It has been pointed out that the oxygen evolving centre of photosystem II utilises four manganese atoms for the oxidation of water to molecular oxygen.[1] The presence of two manganese atoms at the active site of the pseudo-catalase from *Lactobacillus Plantarum* has been demonstrated.[2] Recently, dinuclear manganese(II/II) complexes have been proposed as structural models of the dimanganese catalase enzyme.[3] A manganese(III/III) oxidation state is involved rather than mixed valence states such as $Mn_2^{II,III}$ and $Mn_2^{III,IV}$. On the other hand, di-μ-oxo manganese(III/IV) dimers clearly present similarities with superoxidised *L. Plantarum* manganese catalase.[4]

In this paper, we describe the preparation of various di-μ-oxo manganese(III/IV) complexes and the influence of steric and electronic factors on their activity on H_2O_2 disproportionation in neutral aqueous medium.

Bispicen [*N*,*N*′-bis(2-pyridylmethyl)-1,2-ethanediamine, L1] was prepared as previously described.[5] The 'bispicen' type ligands (L2–8) were synthesised by reacting a diamine with the appropriate chloromethyl derivative in water at room temperature.[6] The preparations of 4-ethoxy-, 4-methyl- and 4-chloro-substituted 2-chloromethyl derivatives were each performed by a three step sequence. The appropriate 2-methylpyridine-1-oxide was treated with acetic anhydride in order to obtain the associated 2-acetoxymethyl derivative. Hydrolysis of the acetyl group and treatment of the resulting alcohol with thionyl chloride afforded the 2-chloromethyl derivatives in satisfactory yields.[7] 4-Nitro-2-chloromethylpyridine was synthesised according to a literature procedure.[8] The di-μ-oxo dimanganese(III/IV) complexes (**1**–**8**) were prepared by classical methods[9] and were identified by element analysis (Fig. 1).†

The X-band EPR spectra, recorded at 4 K, displayed a sixteen hyperfine pattern centred at $g = 2$. Such a spectrum is expected for an antiferromagnetically coupled $Mn^{III/IV}$ dimer with a spin state of $S = \frac{1}{2}$ in which the two manganese ions are not equivalent (localised valence). The cyclic voltammograms consist of two coupled anodic and cathodic waves. In the case of **2** for instance, the first reversible feature centred at 0.22 V (*vs.* SCE) corresponded to the reversible III/III ⟷ III/IV process, and the second peak centred at 1.10 V is attributed to the III/IV ⟷ IV/IV oxidation.[10] The redox potentials of the other complexes increase according to the electronic effect of the *para* substituent on the pyridine ring (Table 1). The potentials of the III/III ⟷ III/IV and III/IV ⟷ IV/IV waves are shifted to more positive values when the hydrogen of bispicen is replaced by methyl **2**, ethyl **7** or isopropyl **8** groups. The effect of the *N*-isopropyl group upon this redox potential is very small compared to the effect of a 6-methyl substituent reported by Hogdson.[10] This allowed us to study the influence of the accessibility of the metal core on the catalytic activity, independently of the influence of the redox potential of the metal centre.

The H_2O_2 disproportionation activity was measured by determination of the time course of oxygen concentration using a Clark type electrode. Simultaneous determination of H_2O_2 concentration was achieved by titration with horseradish peroxidase.‡ Addition of 400 μmol dm^{-3} of H_2O_2 to a deaerated buffered phosphate solution (50 mmol dm^{-3}, pH 7.5, 30 °C) containing 20 μmol dm^{-3} of complex [introduced as a 1 : 1 mixture of manganese(II) perchlorate and the appropriate ligand or the di-μ-oxo(III/IV) complex] initially produced no O_2, but the rate increased to a maximum, and then gradually decreased. This clearly indicates that the active catalyst is formed during the reaction. The lag period during which no oxygen is evolved is very short (a few seconds) and is not observed when a second addition of H_2O_2 is made. Moreover, the initial slope increased and reached a plateau after the third addition. This indicates that the active catalytic species is formed slowly, but accumulates in the solution until a steady



1 R = X = H
2 R = H, X = Me
3 R = OEt, X = Me
4 R = Me, X = Me
5 R = Cl, X = Me
6 R = NO$_2$, X = Me
7 R = H, X = Et
8 R = H, X = Pr^i

Fig. 1

Table 1

	Complex							
	1	2	3	4	5	6	7	8
$E_{1/2}$/V (*vs.* SCE)[a] III/III ⟷ III/IV and III/IV ⟷ IV/IV	0.10 0.81[b]	0.22 1.10	0.11 0.97	0.20 1.09	0.27 1.27	0.30 1.32	0.20 1.14	0.31 1.28
Rate/mol^{-1} s^{-1}[bc]	–[d]	240	140	230	298	352	154	0

[a] Half-wave potential *vs.* SCE corresponding to the III/III ⟷ III/IV and III/IV ⟷ IV/IV processes. [b] See ref. 10. [c] Initial rate constant for H_2O_2 disproportionation in 50 mmol dm^{-3} phosphate buffer (pH 7.5, 30 °C) [complex] = 20 μmol dm^{-3}, $[H_2O_2]_0$ = 400 μmol dm^{-3}. [d] Decomposition occurred during the reaction.

state is reached after three consecutive additions. When the mixture was kept for 30 min after the last addition of H_2O_2, the lag period appeared again when a new amount of H_2O_2 was added. On the other hand this lag period was not present if the mixture is purged with argon and kept for 30 min between two additions, indicating that the active catalyst is O_2 sensitive. Disappearance of the 16-line EPR spectrum after H_2O_2 addition indicated the involvement of EPR silent species (probably III/III or IV/IV dimers). The decrease of the absorbance indicated that the complex was mostly in the III/III oxidation state during the reaction (in the presence of O_2, the initial visible spectrum is recovered after the reaction). We also measured the stoichiometry of the reaction by simultaneous determination of oxygen and H_2O_2 concentrations. During the reaction, the stoichiometry was such that 1 mol of oxygen was produced for every 2 mol of H_2O_2 consumed. Moreover, addition of mannitol (50 mmol dm^{-3}), a very efficient hydroxyl radical trap, does not significantly modify the rate of O_2 formation or the stoichiometry of the reaction. Turnovers as high as 2000 without significant decomposition have been measured for the parent complex **2**. Since most of the previously described manganese catalase analogues are either insoluble or decompose in water, these complexes are interesting catalysts for H_2O_2 disproportionation in neutral aqueous medium.[11] Kinetic studies revealed that the initial rate of oxygen formation $(d[O_2]/dt)_0$ is proportional to the complex concentration (in the range 10–200 μmol dm^{-3} that was used in these experiments) and to the initial concentration of H_2O_2 in the range 0–1 mmol dm^{-3}. Initial rate constants were determined and are collected in Table 1. They were measured in a phosphate buffer when steady state conditions were reached (no variation of the initial slope). The catalytic activity rises with increasing electron-withdrawing character. In the case of derivatives **7** and **8** it dramatically decreased because of the large steric hindrance that prevents the approach of the H_2O_2 molecule.

The catalytic activity is believed to occur *via* the III/III and IV/IV oxidation state. The cucial step for this reaction could be the formation of the active catalyst, either by dismutation or by one electron reduction. During the catalytic process no significant amount of hydroxyl radical is produced, making these complexes very stable in neutral aqueous solution.

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Footnotes

† A procedure taken from ref. 6 rather than that described by Toflund (ref. 9) was used for the preparation of the ligands. Complexes **1** and **2** have been previously described in refs. 9 and 10. $MnCl_2 \cdot 4H_2O$ (1 mmol) was added to 15 ml of distilled water, containing the tetrahydrochloride salt of the ligand (1 mmol) and sodium hydroxide (pH 7–8). A few drops of a hydrogen peroxide solution (30%) were very carefully added with stirring until the solution turned dark olive green. After 30 min, addition of $NaClO_4$ allowed precipitation of the complex which was filtered off and washed with water. The crude products were recrystallized either from hot water or an acetonitrile–dichloromethane mixture to give green–black crystals. The general formula of these complexes was $[(L)MnO]_2(ClO_4)_3$.

Selected data for **3**: Yield 36%. Found: C, 39.80; H, 5.12; Cl 8.78; Mn, 9.12; N, 9.32. $C_{40}H_{60}Cl_3Mn_2N_8O_{18} \cdot 2H_2O$ requires C, 40.25; H, 5.36; Cl, 8.92; Mn, 9.22; N, 9.39.

4: Yield 45%. Found C, 40.97; H, 5.13; Cl, 9.98; Mn, 10.40; N, 10.58. $C_{36}H_{52}Cl_3Mn_2N_8O_{14} \cdot H_2O$ requires C, 40.98; H, 5.15; Cl, 10.08; Mn, 10.40; N, 10.62.

5: Yield 47%. Found C, 34.20; H, 3.64; Cl, 22.01; Mn, 9.76; N, 10.02. $C_{32}H_{40}Cl_7Mn_2N_8O_{14} \cdot H_2O$ requires C, 33.8; H, 3.70; Cl, 21.84; Mn, 9.68; N, 9.86.

6 Yield 53%. Found C, 32.61; H, 3.58; Cl, 8.95; Mn, 9.35; N, 14.21. $C_{32}H_{40}Cl_3Mn_2N_{12}O_{22} \cdot H_2O$ requires C, 32.60; H, 3.59; Cl, 9.02; Mn, 9.32; N, 14.26.

7: Yield 55%. Found C, 40.80; H, 5.18; Cl, 9.92; Mn, 10.23; N, 10.35. $C_{36}H_{52}Cl_3Mn_2N_8O_{14} \cdot H_2O$ requires C, 40.98; H, 5.16; Cl, 10.08; Mn, 10.41; N, 10.62.

8: Yield 51%. Found C, 43.60; H, 5.48; Cl, 9.63; Mn, 9.96; N, 10.20. $C_{40}H_{60}Cl_3Mn_2N_8O_{14} \cdot H_2O$ requires C, 43.59; H, 5.58; Cl, 9.58; Mn, 9.91; N, 10.09.

‡ Horseradish peroxidase (Sigma) was used to oxidise monoelectronically 3,5-dichloro-2-hydroxybenzoic acid (DCHBS), a water soluble phenol. Rapid quenching of the radical by 4-aminoantipyrine (4-AAP) gave a stable chromophore with an absorption maximum at 512 nm (ε = 25000 cm^{-1} dm^3 mol^{-1}). Concentrations: DCHBS 1 mmol dm^{-3}, 4-AAP 250 μmol dm^{-3}, HRP 20 U ml^{-1}; H_2O_2 <40 μmol dm^{-3} in a 100 mmol dm^{-3} phosphate buffer (pH 7.5–8).

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