

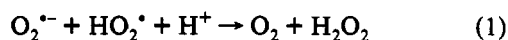
Manganese Macrocyclic Ligand Complexes as Mimics of Superoxide Dismutase

Dennis P. Riley* and Randy H. Weiss

The Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Received August 16, 1993

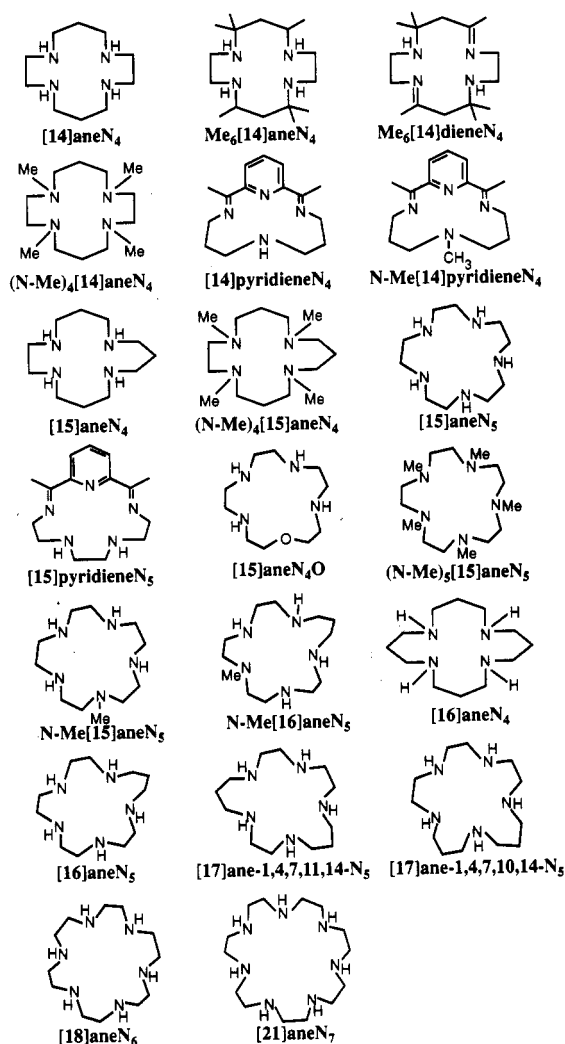
Superoxide radical anion, $O_2^{\cdot-}$ (SO), a product of cellular respiration, activated polymorphonuclear leukocytes, and endothelial cells, has been demonstrated to be a mediator of ischemia reperfusion injury, inflammatory diseases, and vascular diseases.¹ The main line of defense in mammalian organisms for controlling extracellular and intracellular SO are the Cu/Zn- and Mn-containing superoxide dismutase (SOD) enzymes, respectively.² Their role is to efficiently dismutate superoxide to the nonradical products oxygen and hydrogen peroxide (eq 1). The Cu/Zn-



containing enzymes³ catalyze this reaction with pH independent rate constants measured to be in the range $2 \times 10^9 M^{-1} s^{-1}$ while Mn enzymes are ~ 10 -fold less active.⁴ In many of the disease states noted above, SOD enzymes have been observed to provide beneficial effects. Since there are problems (cost, bioavailability, stability, immunogenicity) associated with using an enzyme as a pharmaceutical, we have initiated a program to test in specific disease-state models synthetic low molecular weight SOD mimics as potential pharmaceutical agents.

Herein, we report the discovery of a class of Mn(II) complexes⁵ which demonstrate high SOD activity, chemical stability at physiological pH, and *in vivo* efficacy. We have focused our efforts toward the synthesis of macrocyclic ligand complexes of Mn(II) employing the macrocyclic ligand effect for creating molecules of exceptional kinetic and thermodynamic stability.⁶ In Chart 1 are shown the ligands used to make Mn(II) dichloro complexes.⁷ The complexes were screened in a stopped-flow assay developed for detecting and quantitating SOD activity.^{8,9} Only the Mn(II) complexes of 1,4,7,10,13-pentazacyclopentadecane, [Mn([15]aneN₅)Cl₂], **1**, and 1,4,7,10,13-pentazacyclohexadecane, [Mn([16]aneN₅)Cl₂], **2**, exhibit catalytic SOD activity. The k_{cat} values for the complexes at pH = 8.1 are $2.20 (\pm 0.08) \times 10^7 M^{-1} s^{-1}$ for **1** and $\sim 1.0 \times 10^6 M^{-1} s^{-1}$ for **2**.¹⁰ At physiological

Chart 1. Structures of the Ligands Used To Make [Mn^{II}(L)Cl₂] Complexes That Were Evaluated as SOD Catalysts by Stopped Flow Analysis



pH (~ 7.4), complex **2** shows no detectable SOD activity, while **1** increases in activity: $k_{cat} = 4.13 (\pm 0.10) \times 10^7 M^{-1} s^{-1}$. The loss of SOD activity for **2** as the pH decreases is consistent with the lower acid stability of this complex.^{11,12} The presence of added chloride ion (10 mM) or the presence of other anions, e.g., phosphate, acetate, bromide, perchlorate, and nitrate, has no effect on the catalytic rate of SO dismutation.

Complex **1** was synthesized by reaction of the free base, [15]-aneN₅, with anhydrous MnCl₂ in MeOH under dry N₂. The resultant white complex was recrystallized from EtOH and shown by single-crystal X-ray diffraction to have the seven-coordinate structure depicted in Figure 1.¹³ The complex **1**, is a 1:1 electrolyte in MeOH¹² and exhibits a reversible oxidation at $E_{1/2} = 0.77 V$. Complex **1** rapidly dismutates SO (Figure 2) in a process which is first-order in [SO]. The catalytic rate of loss of SO exhibits a linear dependence on the [H⁺] from pH = 7.4 to 8.6. A [H⁺]

(11) The Mn(H₂O)₆²⁺ species is not a SOD catalyst.⁹

(12) A Mn(II) complex containing the [15]aneN₅ ligand was isolated previously as the [Mn(L)Cl]PF₆ salt, containing a high-spin d⁵ Mn(II) ion which behaves as a 2:1 electrolyte in water and a 1:1 electrolyte in CH₃CN and is stable at pH = 7.4 (log $K_{ML} = 10.7$; Newton, J. E.; Jackels, S. C. *J. Coord. Chem.* 1988, 19, 265); the 16-membered-ring complex, **2**, is $\sim 80\%$ dissociated as monitored by electrospray MS at this pH.

(13) Crystal data of [Mn([15]aneN₅)Cl₂]: monoclinic at 21 °C, space group *Pc-C*₂ (No. 7); $a = 6.968(2) \text{ \AA}$, $b = 7.894(2) \text{ \AA}$, $c = 15.131(4) \text{ \AA}$; $\beta = 102.80(2)^\circ$, $V = 811.5(4) \text{ \AA}^3$, and $Z = 2$ ($\rho_{calc} = 1.396 \text{ g cm}^{-3}$; $\mu_r(\text{Mo K}\alpha) = 1.11 \text{ mm}^{-1}$). See supplementary material.

(1) (a) Bannister, J. V.; Bannister, W. H.; Rotilio, G. *Crit. Rev. Biochem.* 1987, 22, 111. (b) McCord, J. M. *Free Radical Biol. Med.* 1988, 4, 9. (c) McCord, J. M. *J. Free Radical Biol. Med.* 1986, 2, 307. (d) Petkau, A. *Cancer Treat. Rev.* 1986, 13, 17. (e) Hurst, J. K.; Barrette, W. C., Jr. *Crit. Rev. Biochem. Mol. Biol.* 1989, 24, 271. (f) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* 1969, 244, 6049.

(2) Cass, A. E. G. *Superoxide Dismutase. In Metalloproteins. Part 1*; Harrison, P., Ed.; Verlag Chemie: Weinheim, GDR, 1985.

(3) (a) Klug, D.; Rabani, J. *J. Biol. Chem.* 1972, 247, 4839. (b) Rotilio, G.; Bray, R. C.; Fielden, E. M. *Biochim. Biophys. Acta* 1972, 268, 605.

(4) (a) Pick, M.; Rabani, J.; Yost, F.; Fridovich, I. *J. Am. Chem. Soc.* 1974, 96, 7329. (b) McAdam, M. A.; Fox, R. A.; Lavelle, F.; Fielden, E. M. *Biochem. J.* 1977, 165, 71.

(5) We have focused on Mn(II) complexes since Mn(II) ion is much less toxic *in vivo* than copper or iron ions (other metals in SOD enzymes).²

(6) Lindoy, L. F. *The Chemistry of Macrocyclic Ligand Complexes*; Cambridge University Press: Cambridge, Great Britain, 1989.

(7) The syntheses of all the Mn(L)Cl₂ complexes are described in the supplementary material.

(8) Riley, D. P.; Rivers, W. J.; Weiss, R. H. *Anal. Biochem.* 1991, 196, 344.

(9) Weiss, R. H.; Flickinger, A. G.; Rivers, W. J.; Hardy, M. D.; Aston, K. W.; Ryan, U. S.; Riley, D. P. *J. Biol. Chem.* 1993, 268, 23049.

(10) The value of k_{cat} at any pH is obtained from the slope of the plot of k_{cat} versus [Mn(II) complex].⁸ For complex **1** a linear correlation is obtained over a concentration range of $0.8 \times 10^{-6} M$ to $9.0 \times 10^{-6} M$.

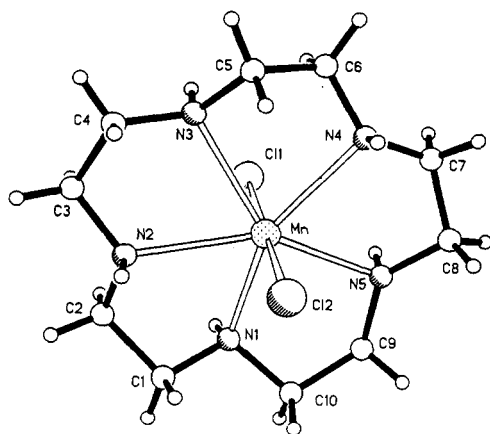
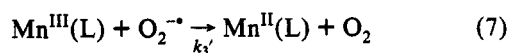
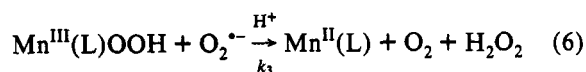
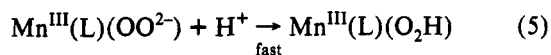
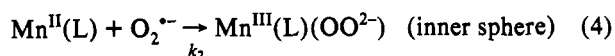
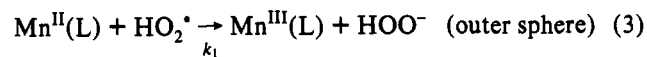


Figure 1. Structure of complex **1**, $[\text{Mn}(\text{[15]aneN}_5)\text{Cl}_2]$, determined by single-crystal X-ray diffraction.

independent pathway is observed ($k_{\text{ind}} = 1.82 \times 10^7 \text{ s}^{-1}$). The observed rate law for dismutation of SO is shown in eq 2. The

$$V = -d[\text{O}_2^{\cdot-}]/dt \propto [\text{Mn}]_{\text{tot}}^1 [\text{O}_2^{\cdot-}]^1 \{k_{\text{H}^+}[\text{H}^+]^1 + k_{\text{ind}}\} \quad (2)$$

measured value of k_{H^+} at 21 °C is $0.58 \times 10^{15} \text{ M}^{-1} \text{ s}^{-1}$. Complex **1** does not react with H_2O_2 ; and under catalytic conditions (i.e., $[\text{O}_2^{\cdot-}] > [\text{1}]$), there is no detectable oxidized Mn species present; i.e., $[\text{Mn}]_{\text{tot}} = [\text{Mn}^{\text{II}}(\text{L})]$.¹⁴ The data are consistent with the mechanistic scheme summarized in eqs 3–7. Assuming $[\text{Mn}]_{\text{tot}}$



$= [\text{Mn}^{\text{II}}(\text{L})]$, the integrated rate expression derived for this scheme (eq 8) is consistent with the observed kinetics, where K_a is the acidity constant for HO_2^{\cdot} .¹⁵

$$V = 2[\text{Mn}]_{\text{tot}}[\text{O}_2^{\cdot-}]\{k_2 + k_1[\text{H}^+]/K\} \quad (8)$$

A pH dependent process (eq 3) is consistent with the oxidation of **1** with HO_2^{\cdot} via an outer-sphere path to afford an intermediate Mn^{III} complex (at pH = 7.4, $k_1[\text{H}^+]/K_a = 1.16 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$). The formation of $\text{Mn}^{\text{III}}(\text{L})(\text{OO}^{2-})$, **3**, depicted in eq 4 as the pH independent path, is analogous to the reaction of the complex (tetraphenylporphyrinato)manganese(II) with SO in which a

(14) Complex **1** was oxidized to its Mn^{III} complex in water using cerium(IV). The complex shows an absorption maximum at $\lambda_{\text{max}} = 448 \text{ nm}$ ($\epsilon = 1430$). This $\text{Mn}(\text{III})$ complex is stable in anhydrous Me_2SO , but is reduced back to **1** by a variety of reducing agents including alcohols.

(15) $\text{p}K_a(\text{HO}_2^{\cdot}) = 4.69$. Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1981**, *14*, 393.

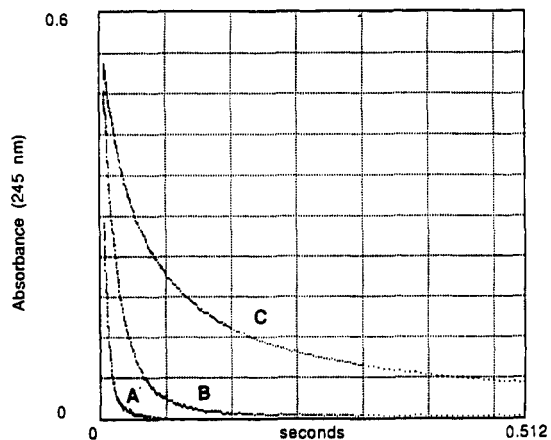


Figure 2. Stopped-flow decays of superoxide measured at 245 nm at 21 °C, where $[\text{SO}]_i \sim 100 \mu\text{M}$, in the presence and absence of complex **1**. All reactions were carried out using 80 mmol of HEPES buffer at pH = 7.75. A: $8.80 \times 10^{-6} \text{ M}$ $[\text{Mn}(\text{[15]aneN}_5)\text{Cl}]\text{Cl}$. B: $2.20 \times 10^{-6} \text{ M}$ $[\text{Mn}(\text{[15]aneN}_5)\text{Cl}]\text{Cl}$. C: uncatalyzed.

Mn^{III} side-on bonded peroxy species is formed.¹⁶ Reaction of **1** with KO_2 in anhydrous Me_2SO generates a species whose electronic spectrum is similar to the spectrum of $\text{Mn}^{\text{III}}(\text{L})$,¹⁴ but the presence of protic material results in its rapid (<millisecond) decay (eq 5). This reaction (eq 4) is limited to a maximum rate governed by the rate of ligand exchange on the axial sites of the Mn complex. Accordingly, the dissociative exchange of water on the (hexaquo)manganese(II) ion has been measured at pH = 7 to be $\sim 0.8 \times 10^7 \text{ s}^{-1}$ at 25 °C;¹⁷ and the measured value of k_2 ($0.91 \times 10^7 \text{ s}^{-1}$) is in good agreement with this value.

The complex **1**, $[\text{Mn}(\text{[15]aneN}_5)\text{Cl}_2]$, represents the first example of a well-defined Mn^{II} complex that is an excellent SOD mimic and also is stable to dissociation and hydrolysis at physiologically relevant pH. Complexes derived from **1** containing C-bonded substituents on the macrocyclic ring are currently being investigated and are observed in some instances to enhance the SOD activity of the Mn center.¹⁸ Finally, **1** has been studied extensively in an *in vivo* model for myocardial infarct and has been found to be an effective agent for reducing the superoxide-mediated injury resulting from reperfusion following ischemia.¹⁹

Acknowledgment. We thank Mr. Karl W. Aston and Willie J. Rivers for their excellent technical assistance and thank Profs. D. H. Busch, J. T. Groves, and J. Halpern for their stimulating discussions.

Supplementary Material Available: Crystallographic details for $\text{Mn}(\text{[15]aneN}_5)\text{Cl}_2$, **1**, and experimental procedures and spectral details for the $\text{Mn}(\text{II})$ dichloro complexes of the compounds shown in Chart 1 (23 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(16) (a) Valentine, J. S.; Quinn, A. E. *Inorg. Chem.* **1976**, *15*, 1997. (b) VanAtta, R. B.; Strouse, C. E.; Hanson, L. K.; Valentine, J. S. *J. Am. Chem. Soc.* **1987**, *109*, 1425.

(17) Eigen, M. *Pure Appl. Chem.* **1963**, *6*, 105.

(18) Studies in progress.

(19) Friedrichs, G. S.; Kilgore, K. S.; Chi, L.; Ryan, U. S.; Lucchesi, B. R. *FASEB J.* **1993**, *7*(4), 2457.