Superoxide Dismutase Mimetic Complex of Mn(II) / *N*, *N*-Bis(2-Pyridylmethyl)-(*S*)-Histidine

Kazuhiro Yamato, Ikuko Miyahara, Akio Ichimura, Ken Hirotsu, Yoshitane Kojima,* Hiromu Sakurai,[††]
Daisuke Shiomi,[†] Kazunobu Sato, and Takeji Takui
Department of Chemistry, Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558-8585
[†]*Department of Material Science, Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558-8585*
[††]*Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University,*
5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414

(Received December 7, 1998; CL-980903)

A novel dinuclear Mn(II) complex, $[Mn(L)(H_2O)]_2(ClO_4)_2$ (**1**) where L is *N*, *N*-bis(2-pyridylmethyl)-(*S*)-histidinate, was synthesized. X-ray structure analysis of **1** revealed that each Mn(II) ion is a doubly carboxylate-bridged sevencoordinate structure. Cyclic voltammmetry indicated that electrode reaction of **1** was dependent on pH. The reactivity toward superoxide was evaluated by *in vitro* xanthine-xanthine oxidase-cytochrome *c* assay. The IC_{50} value of **1** is equal to 0.29 μM (1 M = 1 mol dm^{-3}), which is one of the highest values in Mn(II) SOD mimetic complexes.

Superoxide dismutases (SODs), which contain either Cu-Zn, Mn or Fe ion at the active site, catalyze the dismutation of superoxide anion radical ($O_2^{\cdot-}$) to hydrogen peroxide and molecular oxygen.[1]

$$2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$$

They play important roles in the protection of living organisms against active oxygen species, because $O_2^{\cdot-}$ is supposed to, in part, mediate several disease states such as tissue inflammation, cancer and AIDS.[2] However, SOD enzymes have only limited therapeutic applications because of lack of oral activity and short half lives. Thus, synthetic low molecular weight SOD mimetic complexes have been reported.[4] Herein, we report a novel Mn(II) / *N*, *N*-bis(2-pyridylmethy)-(*S*)-histidinate (L) complex as a SOD mimetic complex.

The ligand was prepared in L methyl ester form by our previous method.[5] After hydrolysis of L methyl ester with barium hydroxide in an aqueous methanol solution, manganese(II) sulfate was added, followed by a counter anion exchange with barium perchlorate.[6]

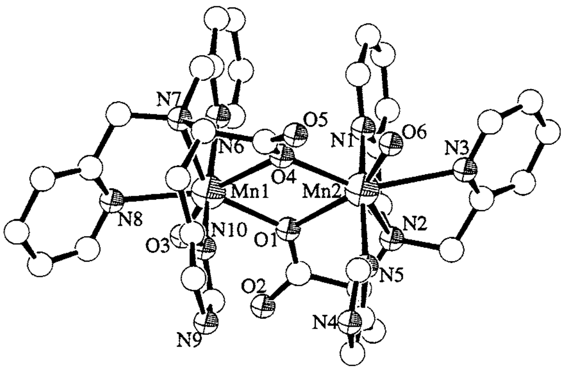

Figure 1. ORTEP drawing of **1**. Hydrogen atoms, perchlolate anions, and solvate water molecules are omitted for clarity. Selected bond lengths (Å): Mn(1)-O(1) = 2.224(1), Mn(1)-O(3) = 2.205(4), Mn(1)-O(4) = 2.464(5), Mn(1)-N(6) = 2.25(2), Mn(1)-N(7) = 2.4007(9), Mn(1)-N(8) = 2.397(1), Mn(1)-N(10) = 2.222(4), Mn(2)-O(1) = 2.370(1), Mn(2)-O(4) = 2.287(4), Mn(2)-O(6) = 2.199, Mn(2)-N(1) = 2.203(5), Mn(2)-N(2) = 2.35(2), Mn(2)-N(3) = 2.644(7), Mn(2)-N(5) = 2.17(1), Mn(1)-Mn(2) = 3.793(2).

X-Ray structure analysis shows that **1** is a doubly carboxylate bridged asymmetric dinuclear structure in the solid state (Figure 1).[7] Each Mn(II) ion binds to N_4O donor atoms on the bases of ligand L, one bridged carboxylate oxygen of another ligand L and one water molecule. As a result, each coordination geometry of Mn(II) is a distorted pentagonal bipyramidal, with two nitrogen atoms at the axial positions. In addition, the $Mn\text{-}(O)_2\text{-}Mn$ core locates at a near plane (the torsion angle is 173°).

Magnetic susceptibility measurements of complex **1** in the polycrystalline state by SQUID in the range of 1.7 to 300 K showed a weak antiferromagnetic coupling between two Mn(II) ions with $J/k_B = -1.0$ K (dimer model) and $\mu_{eff} = 8.35\ \mu_B$ ($g \sim 2.00$). X-band ESR measurements of complex **1** in the polycrystalline state at 3 K revealed a broadened fine-structure spectrum, reflecting low symmetry of the high spin site and fine-structure constants. ESR spectra of **1** in H_2O at ambient temperature showed mononuclear hyperfine features from Mn(II) ions.

Figure 2 shows the cyclic voltammograms of **1** at a glassy carbon electrode in 50mM HCl-γ-collidine buffer (pH 7.5) with various scan rates. Complex **1** showed chemically irreversible one oxidation wave (E_{pa} ca. 500 mV) and two re-reduction waves (E_{pc1} ca. 250 mV and E_{pc2} ca. −20 mV). At faster scan rate, the peak at E_{pc1} decreased, while that at E_{pc2} increased.

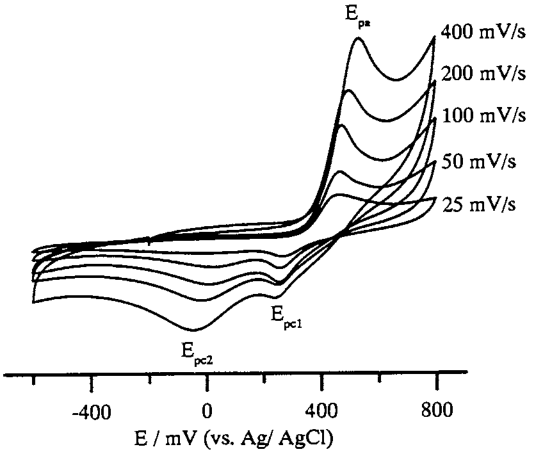

Figure 2. Cyclic voltammograms of **1** in HCl-γ-collidine buffer (pH 7.5) with various scan rate.

This result suggests that oxidation of **1** causes structure changes. This redox process is dependent on pH, and the E_{pa} value changes linearly with a slope of −65mV (Figure 3). At lower pH, the rate of protonation is faster, so the reversibility is more

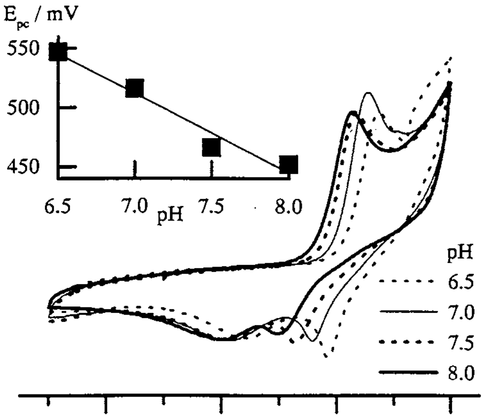

Figure 3. The pH dependent cyclic voltammograms of **1**, inside: The pH dependent cathodic peak potential (Epc).

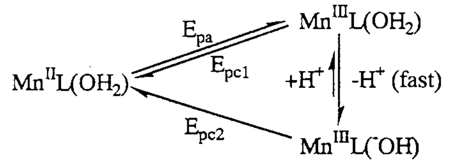

Figure 4. The proposed electrode reaction mechanisms for **1**.

improved. Thus, the electrode reaction mechanism for the complex **1** includes the deprotonation/protonation of the coordinated water molecule (Figure 4). Furthermore, these potential values exist between -560 (E^0 O_2/O_2^-) and +650 (E^0 O_2^-/H_2O_2) mV (vs. Ag/AgCl).[8] The above results suggest that the complex **1** is capable of serving as a SOD mimetic complex.

The SOD like activity of **1** was evaluated by *in vitro* xanthine-xanthine oxidase-cytochrome *c* assay.[9] Complex **1** shows an IC_{50} value of 0.29 μM (vs. Mn(II) ion) which indicates that **1** serves as an efficient SOD mimetic complex. Furthermore, the value is comparable to those of some manganese complexes with the lowest value (0.30-0.80 μM).[11]

In conclusion, we propose here about a new highly active SOD mimetic Mn(II) complex. The present results are useful for designing and developing new SOD mimetic complexes with high activity. In addition, the relationships between SOD like activities and structure factors, such as charges and coordination numbers of complexes, are quite interesting. Further research in this line is now under way.

The authors are grateful to Ms. Tamaki Nagasawa for elemental analysis. This work was partially supported by the Osaka City as the Special Fund for the Student of the Graduate School of Science, Osaka City University.

References and Notes

1 J. M. McCord and I. Fridovich, *J. Biol. Chem.*, **244**, 6049 (1969).
2 B. K. Shina and E. G. Mimnaugh, *Free. Rad. Biol. Med.*, **8**, 567 (1990).
3 M. T. Droy-Lefaix, Y. Drouet, G. Geraud, D. Hosfold, and P. Braquet, *Free Rad. Res. Commun.*, **12-13**, 725 (1991).
4 a) T. Nagano, T. Hirano, and M. Hirobe, *Free Rad. Res. Commun.*, **12-13**, 221 (1991). b) C. Itami, H. Matsunaga, T. Sawada, H. Sakurai, and Y. Kimura, *Biochem. Biophys. Res. Commun.*, **197**, 536 (1993). c) K. Wada, Y. Fujibayashi, and A. Yokoyama, *Arch. Biochem. Biophys.*, **310**, 1 (1994). d) R. H. Weiss and D. P. Reley, *Drugs Future*, **21**, 383 (1996).
5 K. Kawabe, T. Suekuni, T. Inada, K. Yamato, M. Tadokoro, Y. Kojima, Y. Fujisawa, and H. Sakurai, *Chem. Lett.*, **1998**, 1155.
6 The aqueous mehtanol solution (1/1) of L methyl ester (0.35 g, 1.0 mmol) and $Ba(OH)_2 \cdot 8H_2O$ (0.16 g, 0.50 mmol) was stirred for 8 h. Then, $MnSO_4 \cdot 5H_2O$ (0.24 g, 1.0mmol) and $Ba(ClO_4)_2 \cdot 8H_2O$ (0.20 g, 0.50 mmol) were added. After removal of the precipitation, the filtrate was evaporated slowly. White microcrystals were precipitated. Yield 51%. Anal. Found: C, 40.99; H, 4.12; N, 13.29%. Calcd. for $[Mn(C_{18}H_{18}N_5O_2)]_2(ClO_4)_2 \cdot 4H_2O$: C, 41.04; H, 4.21; N, 13.29%. m.p. 215-230 °C (dec.). $[\alpha]_D$ = -15.5 deg dm^{-1} g^{-1} cm^3 (methanol). IR (KBr) 1602 ($\nu_{C=O}$), 1094 ($\nu_{perchlorate}$).
7 White single crystal (0.40 × 0.30 × 0.08 mm) was obtained by recrystalization from H_2O / EtOH (10/1). Crystal data for **1**: $C_{36}H_{46}N_{10}O_{17}Cl_2Mn_2$, M = 1071.60, monoclinic, space group $P2_1$(#4), a = 8.699(4), b = 11.937(3), c = 23.305(4) Å, β = 92.49(3)°, V = 2417(1) Å^3, F(000) = 1004.00, Z = 4, Dc = 1.46 g/cm^3, μ(Mo-Kα) = 7.10 cm^{-1}, T = 296 K, $2\theta_{max}$ = 60.0°. Intensity data (7807 total reflections, 7364 unique reflections) were collected on Rigaku AFC7R diffractometer. The final cycle of full-matrix least squares refinement was based on 4924 observed reflections (I >3.00 $\sigma(I)$) and 595 variable parameters, and converged to R = 0.075 and Rw = 0.101. Maximum peak in final differential map is 1.57 eÅ^{-3}. Programs used to solve structure: SIR92. All calculations including data reduction: teXsan crystallographic software package (Molecular Structure Corporation).
8 W. H. Koppenol, *Nature*, **262**, 420 (1976).
9 C. Beauchamp and I. Fridovich, *Anal. Biochem.*, **44**, 276 (1971). Superoxide anions were generated *in situ* by xanthine–xanthine oxidase system and detected spectrophotometricaly by reduction of ferricytochrome *c*. The assays were carried out in γ-collidine buffer (50 mM, pH 7.8, 25.0 °C) containing 10 μM ferricytochrome *c*, 50 μM xanthine and an amount of xanthine oxidase to cause a change of absorbance (ΔA_{550} = 0.025 min^{-1}). The IC_{50} values are defined as the 50% inhibition concentration of cytochrome *c* reduction. Before the assay, we have examined whether Mn(II) and its complexes used in the present investigation inhibit xanthine oxidase by use of the maximum at 290 nm due to uric acid at pH 7.8 as reported,[10] and concluded that they don't inhibit xanthine oxidase under the experimental conditions.
10 a) N. Cotell, J. L. Bernier, J. P. Henichart, J. P. Catteau, E. Gaydou, and J. C. Wallet, *Free Rad. Biol. Med.*, **13**, 221 (1992). b) W. S. Chang, Y. J. Lee, F. J. Lu, and H. C. Chiang, *Anticancer Res.*, **13**, 2165 (1993).
11 a) M. Baudry, S. Etienne, A. Bruce, M. Palucki, E. Jacobsen, and B. Halliwell, *Biochem. Biophys. Res. Commun.*, **192**, 964 (1993). b) N. Kitajima, M. Osawa, N. Tamura, Y. Moro-oka, T. Hirano, M. Hirobe, and T. Nagano, *Inorg. Chem.*, **32**, 1879 (1993). c) D. F. Xiang, C. Y. Duan, X. S. Tan, Q. W. Hang, and W. X. Tang, *J. Chem. Soc., Dalton Trans.*, **1998**, 1201.