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Monomeric (Benzoato)manganese(II) Complexes as Manganese Superoxide Dismutase Mimics

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Superoxide dismutase (SOD) catalyzes the dismutation of superoxide ion (O_2^-)[1] and protects living cells against the toxicity of hyperoxia and against the dioxygen-dependent toxicities[2] of viologens, quinones, hypervalent compounds, and benzofurazans. Recently, the application of SOD as a pharmaceutical has attracted much attention,[3] because superoxide has been reported to cause some diseases such as inflammation, ischemia damage, and cancer. A stable, nontoxic, low-molecular-weight metal complex[4,5] that catalyzes the dismutation of superoxide ion might be able to substitute for SOD in such applications, with desirable qualities being low cost, cell permeability, and nonimmunogenicity. As a part of our synthetic endeavors[6] with a hindered tris(pyrazolyl)borate ligand $HB(3,5\text{-}iPr_2pz)_3$,[7,8] we have now succeeded in preparing manganese complexes which bear structural similarities to the active site of Mn–SOD[9] and found that the complexes are effective for superoxide dismutation.

Anaerobic reactions of $Mn(Cl)(HB(3,5\text{-}iPr_2pz)_3)$ and $Mn(Cl)(3,5\text{-}iPr_2pzH)(HB(3,5\text{-}iPr_2pz)_3)$ with NaOBz gave manganese(II) complexes $Mn(OBz)(HB(3,5\text{-}iPr_2pz)_3)$ (**1**)[10] and $Mn(OBz)(3,5\text{-}iPr_2pzH)(HB(3,5\text{-}iPr_2pz)_3)$ (**2**),[11] respectively. Complex **2** was also preparable by the addition of 1 equiv of 3,5-iPrpzH to **1**, whereas the conversion of **2** to **1** was unsuccessful owing to the strong affinity of $3,5\text{-}iPr_2pzH$ for the manganese(II) ion. Complex **2** is air stable, while **1** is slightly oxygen-sensitive. The molecular structure of **2** was determined by X-ray crystallography.[12] Given in Figure 1 is an ORTEP view of **2**. Complex **2** possesses a monomeric structure with a N_4O ligand donor set. The benzoate group is coordinated to the manganese unidentately. The nonliganding oxygen atom (O2) of the benzoate

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(7) Abbreviations used: $HB(3,5\text{-}iPr_2pz)_3$ = hydrotris(3,5-diisopropyl-1-pyrazolyl)borate, $3,5\text{-}iPr_2pzH$ = 3,5-diisopropylpyrazole, PA = picolinato, TPEN = *N,N,N',N'*-tetrakis(2-pyridylmethyl)ethylenediamine.

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(10) Complex **1** was obtained by the reaction of $Mn(Cl)(HB(3,5\text{-}iPr_2pz)_3)$ with 1.5 equiv of NaOBz in a mixture of toluene and MeCN (4:1) in ca. 60% yield. Anal. Calcd for $C_{34}H_{51}N_6BO_2Mn$: C, 63.65; H, 8.01; N, 13.10. Found: C, 63.91; H, 7.73; N, 13.06. IR (cm^{-1}): ν(BH), 2539; ν(C=C), 1611; ν_a(COO), 1568; ν_s(COO), 1430. FD–MS (*m/e*): 642. The X-ray structure of **1** was refined to the current R (R_w) factor of 5.54% (6.49%) for 3874 independent reflections; Mn–O1 = 2.206(4) Å and Mn–O2 = 2.125(4) Å. The details will be reported elsewhere.

(11) Complex **2** was prepared by the reaction of $Mn(Cl)(3,5\text{-}iPr_2pzH)(HB(3,5\text{-}iPr_2pz)_3)$ with 1 equiv of NaOBz in 75% yield. Anal. Calcd for $C_{43}H_{67}N_8BO_2Mn$: C, 65.06; H, 8.51; N, 14.12. Found: C, 65.33; H, 8.27; N, 14.07. IR (cm^{-1}): ν(BH), 2530; ν(C=C), 1600; ν_a(COO), 1555; ν_s(COO), 1438. FD–MS (*m/e*): 794.

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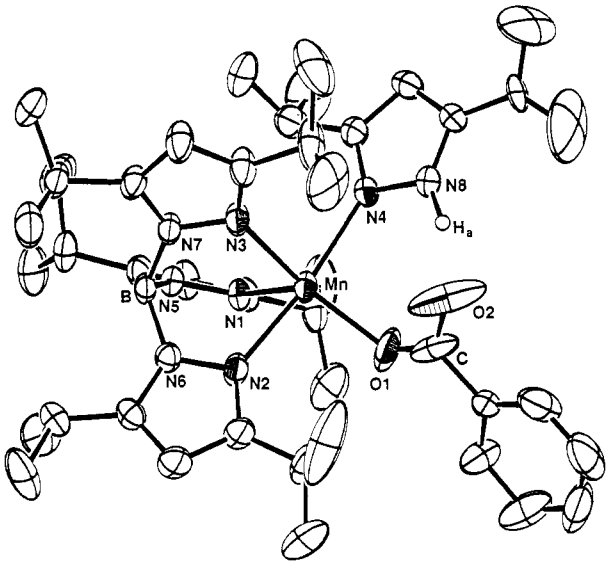

Figure 1. ORTEP view of Mn(OBz)(3,5-iPr_2pzH)(HB(3,5-iPr_2pz)$_3$) (**2**). Selected bond distances (Å) and angles (deg): Mn–N1, 2.195(4); Mn–N2, 2.277(4); Mn–N3, 2.161(3); Mn–N4, 2.304(4); Mn–O1, 2.043(4); C–O1, 1.190(8); C–O2, 1.263(7); O2–N8, 2.684(6); H_a–N8, 1.04(4); H_a–O2, 1.67(4); N2–Mn–N4, 171.2(1); O1–Mn–N1, 153.8(2); O1–Mn–N3, 111.3(2); N1–Mn–N3, 94.3(1).

forms a hydrogen bond with the proton (H_a) on 3,5-iPr_2pzH. The existence of the hydrogen bond is clearly established on the basis of the following evidence: (1) The hydrogen atom is located from the X-ray structure with mean bond distances of H_a–O2 = 1.67(4) Å and H_a–N8 = 1.04(4) Å. (2) There is a short distance for the O2–N8 bond (2.684(6) Å). (3) No clear band due to ν(NH) is observed in the IR spectrum (free 3,5-iPr_2pzH gives the band[13] at 3180 cm^{-1}). The coordination geometry of **2** may be best described as distorted trigonal-bipyramidal with N2 and N4 as apical ligands. Thus, the structural features of **2** are close to those known for the active site of Mn–SOD.[9] The manganese in Mn–SOD adopts a trigonal-bipyramidal coordination geometry with a N_3O_2 ligand donor set. One histidyl nitrogen occupies the apical position while water is suggested to sit at the opposite site. The carboxylate oxygen from aspartate is bound to the manganese unidentately as in the case of **2**, constructing a basal plane with two other histidyl nitrogen atoms. The nonliganding oxygen from the aspartate forms a hydrogen bond with an amino acid residue or the peptide backbone.[9]

The IR spectrum of **1**[10] suggested the bidentate coordination of benzoate to the manganese. This was definitely confirmed by the X-ray analysis, which established the five-coordinate structure with a N_3O_2 ligand donor set for **1**.[10]

(12) **2** (fw 793.80) crystallized (recrystallized from MeCN) in the triclinic space group $P\bar{1}$ with a = 13.653(3) Å, b = 16.250(4) Å, c = 13.325(4) Å, α = 105.72(3)°, β = 118.78(2)°, γ = 96.41(2)°, V = 2389(1) Å^3, Z = 2, and D_c = 1.10 g·cm^{-3}. The structure was solved by direct methods (MITHRIL) and refined by the full-matrix least-squares technique with TEXSAN. The location of the hydrogen atom (H_a) was found in the difference Fourier map when all non-hydrogen atoms were refined anisotropically, and it was refined isotropically. The other hydrogens were calculated and fixed in the final refinement cycles. The final R (R_w) factor was 5.39% (3.80%) for 4512 reflections ($3° \le 2\theta \le 50°$, $F_o \ge 6\sigma F_o$).

(13) The complex which contains non-hydrogen-bonding 3,5-iPr_2pzH, Mn(Cl)(3,5-iPr_2pzH)(HB(3,5-iPr_2pz)$_3$), exhibits ν(NH) at 3292 cm^{-1}.

(14) A bidentate benzoato complex, Fe(OBz)(MeCN)(HB(3,5-iPr_2pz)$_3$),[6c] gives ν_a(COO) at 1537 cm^{-1} and ν_s(COO) at 1418 cm^{-1}, whereas a unidentate acetato complex, Zn(OAc)(HB(3,5-iPr_2pz)$_3$),[15] gives ν_a(COO) at 1601 cm^{-1} and ν_s(COO) at 1331 cm^{-1}.

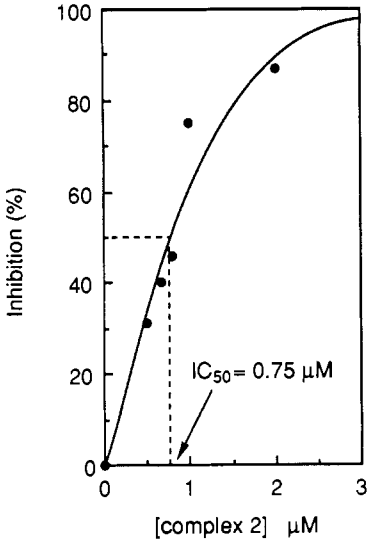

Figure 2. SOD activity of complex **2** in the xanthine oxidase–NBT assay.

We examined the SOD activities of **1** and **2** using the xanthine–xanthine oxidase–nitro blue tetrazolium (NBT) method. The NBT assay was performed in 3 mL of 50 mM potassium phosphate buffer (pH 7.4) at 25 °C in the absence of EDTA. The reaction mixture contained 50 μM NBT, 50 μM xanthine, 1000 U/mL catalase, and 0.04 U/mL xanthine oxidase, to produce about 1.2 μM/min of superoxide ion. The formation of diformazan was followed at 560 nm. The IC_{50} value in Figure 2 means the concentration of the complex which exerts the SOD activity equivalent to one unit of native SOD. The IC_{50} values of **1** and **2** were 0.8 μM (data not shown) and 0.75 μM (Figure 2), respectively, which indicate that these complexes are potent SOD mimics. To ascertain the effectiveness of the present complexes as functional SOD mimics, we compared the SOD activity of $Mn^{II}(PA)_2(PAH)(H_2O)$, which was previously demonstrated as a SOD model,[4b] by the NBT method under the same conditions. The activity of the complex (IC_{50} = 6.5 μM) is distinctively lower than those of **1** and **2**. The much lower activity of $[Mn^{II}(TPEN)]^{2+}$ (IC_{50} = 35 μM)[16] also supports the superior SOD activities of the present complexes, which may be attributable to the structural similarities of these complexes to the active site of Mn–SOD.

Diformazan prepared by $Na_2S_2O_4$ was not reoxidized in the superoxide-generating system in the presence of **1** or **2**. This confirms that these complexes have the activity of SOD but not that of diformazan superoxidase or that of diformazan peroxidase.

Further studies on the therapeutic application of **1** and **2** and other related complexes are now in progress.

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Supplementary Material Available: Text giving X-ray procedures, tables listing crystal data, atomic coordinates and temperature factors, hydrogen atom coordinates, and intramolecular bond distances and angles, and a figure showing an ORTEP plot for **2** (9 pages). Ordering information is given on any current masthead page.

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