DALTON COMMUNICATION

Synthesis, characterisation and superoxide dismutase activity of a manganese(II) complex

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A potent superoxide dismutase mimic, $Mn^{II}(HL)_2$ [H_2L = 2,6-bis(benzimidazol-2-yl)pyridine] has been synthesised and characterised by its crystal structure determination and EPR spectroscopy.

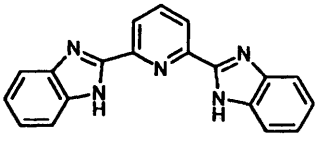
The superoxide radical anion, a product of cellular respiration, activated polymorphonuclear leukocytes and endothelial cells has been demonstrated to be a mediator of ischemia, reperfusion injury, inflammatory and vascular diseases.[1] The main line of defence in mammalian organisms for controlling extracellular and intracellular superoxide radical anions are the Cu–Zn, Mn and Fe containing superoxide dismutase enzymes.[2] Superoxide dismutase catalyses the dismutation of superoxide radical anions to the non-radical products oxygen and hydrogen peroxide[3] [equation (1)] and protects the living cells against the toxicity of hyperoxia.

$$O_2^{\cdot -} + HO_2^{\cdot} + H^+ \longrightarrow O_2 + H_2O_2 \qquad (1)$$

The application of superoxide dismutase as a pharmaceutical has attracted some attention.[4–6] A stable non-toxic, low molecular weight metal complex that catalyses the dismutation of superoxide anion might be a suitable alternative to superoxide dismutase in clinical applications,[7,8] with the desirable qualities of low cost, cell permeability and non-immunogenicity. We have attempted to develop manganese-based superoxidase mimics because copper(II) and iron(III) complexes have the potential of participating in the Fenton reaction with the production of $HO^{\cdot}$, whereas manganese-(II) and -(III) complexes do not do so. The crystal structure of the native (oxidised form) Mn–superoxide dismutase reveals that the manganese adopts a trigonal-bipyramidal co-ordination geometry with N_3O_2 ligand-donor sets,[9] the three nitrogens being provided by three histidine residues in the protein. Many of the manganese complexes reported to show superoxide dismutase activity however, do not possess trigonal-bipyramidal geometry,[10–12] indeed one active manganese(II) compound exhibits seven-co-ordination.[12] Different ligand environments in which manganese-(II) or -(III) is known to exhibit superoxide dismutase-type activity include Schiff bases[10d] and a 15-membered macrocycle.[12] In this communication we describe the synthesis, characterisation and superoxide dismutase-type activity of a manganese(II) complex of the tridentate ligand H_2L providing an N_3-co-ordination environment. We were prompted to study the co-ordination chemistry of H_2L because of the opportunity for the formation of MnN_3X_2 molecules.

2,6-Bis(benzimidazol-2-yl)pyridine (H_2L) was prepared from *o*-phenylenediamine and pyridine-2,6-dicarboxylic acid according to a published procedure.[13] Dissolution of H_2L (6.4 g, 0.02 mol) in warm methanol (100 cm^3) was then followed by addition of $MnCl_2 \cdot 4H_2O$ (1.9 g, 0.01 mol) and the mixture was refluxed for 10 min. The solution on cooling deposited yellow crystals which were recrystallised from methanol–dimethyl sulfoxide–ethyl acetate (1:1:1).† X-Ray quality crystals were grown from dimethyl sulfoxide–ethyl acetate–water (1:1:0.5) and a crystal of dimensions 0.13 × 0.15 × 0.33 mm was used for crystal structure determination.‡

Manganese(II) has previously been shown to form a polymeric seven-co-ordinate complex with a tridentate ligand with three co-ordinating nitrogens particularly when chloride is the counter ion.[15] Here, irrespective of the metal–ligand ratio used in the synthetic procedure, the same monomeric product was always obtained. No chloro-bridged products could be isolated. An ORTEP[16] projection of the compound with the atomic numbering is shown in Fig. 1. The complex is clearly monomeric with no chloride in the manganese co-ordination sphere. The structure contains one manganese and two HL^- with two solvent molecules (ethyl acetate and water). It is interesting that the ligand co-ordinates as a uninegative anionic ligand, for even though a base was not used in the synthetic procedure, N(1) and N(6) are deprotonated with Mn–N(1) and Mn–N(6) 2.245(5) and 2.224(5) Å, respectively. The corresponding protonated nitrogens as expected form marginally longer bonds with Mn–N(4) 2.272(6) and Mn–N(9) 2.330(7) Å, respectively. The co-ordination geometry shows considerable distortion from a regular octahedron as is evident from the bond angles around manganese; N(8)–Mn–N(9) is considerably smaller than the ideal 90°. The metal–nitrogen bond lengths are typical of those expected for Mn^{II}–N bonds.



H_2L

† Found: C, 65.55; H, 3.80; Mn, 7.65; N, 20.40. Calc. for $C_{38}H_{24}MnN_{10} \cdot H_2O$: C, 65.80; H, 3.45; Mn, 7.95; N, 20.0%.

‡ Crystal data. $C_{38}H_{24}MnN_{10} \cdot C_4H_8O_2 \cdot H_2O$, M = 781.73, monoclinic, space group Cc (no. 9), a = 13.644(1), b = 15.309(2), c = 18.558(3) Å, β = 110.28(1)°, U = 3636(1) Å^3, Z = 4, D_c = 1.43 g cm^{-3}, μ(Mo-Kα) = 4.02 cm^{-1}, $F(000)$ = 1620, T = 16 °C, $R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o| = 0.0484$, $R' = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{\frac{1}{2}} = 0.0488$, $w = (\sigma^2|F_o|)^{-1}$, for 2588 observed reflections [$I > 2.5\sigma(I)$]. Data were collected on an Enraf-Nonius CAD4 diffractometer equipped with graphite-monochromated Mo-Kα radiation (λ = 0.7107 Å) and employing the ω–2θ scan mode; 2θ range 2–50°. They were corrected for Lorentz-polarisation effects as well as absorption. The structure was solved by direct methods using the SHELX suite of programs.[14] This gave the position of the Mn and the co-ordination sphere. The remaining atoms were located from successive Fourier-difference maps. Atomic coordinates, thermal parameters and bond lengths and angles have been deposited at the Cambridge Crystallogaphic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1996, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 186/34.

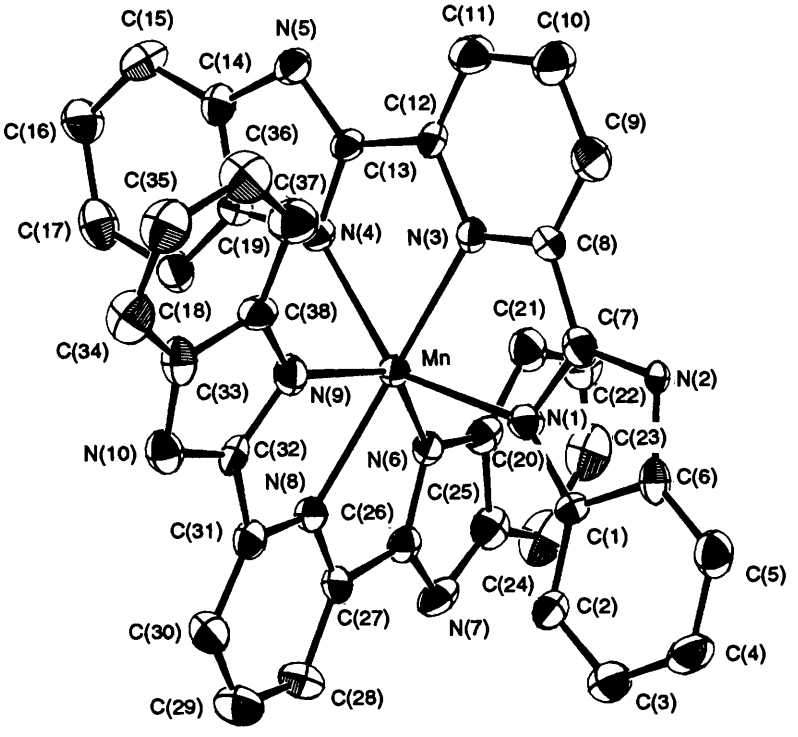


Fig. 1 Structure of $Mn(HL)_2$ with selected bond lengths (Å) and angles (°): Mn–N(1) 2.245(5), Mn–N(3) 2.224(7), Mn–N(4) 2.272(6), Mn–N(6) 2.224(5), Mn–N(8) 2.228(7) and Mn–N(9) 2.330(7); N(1)–Mn–N(3) 72.4(2), N(1)–Mn–N(4) 144.1(2), N(1)–Mn–N(6) 98.8(2), N(3)–Mn–N(6) 123.1(2), N(3)–Mn–N(8) 162.3(2), N(6)–Mn–N(8) 72.6(2) and N(8)–Mn–N(9) 71.4(2)

The room-temperature magnetic moment of a dimethyl sulfoxide solution of the complex, determined using the Evans method,[17] was 5.85 μ_B ($\mu_B \approx 9.274\,02 \times 10^{-24}$ J T^{-1}), which is close to the value expected for high-spin Mn^{II}.

The X-band EPR spectrum of the complex at 300 K exhibits a signal at $g \approx 2$ with prominent fine-structure and no resolved hyperfine structure from ^{55}Mn. The spectrum in dimethyl sulfoxide at 77 K (63 mW microwave power, 2.5 G modulation amplitude, 9.3 GHz) clearly shows that in this solvent the complex exists as a monomeric unit in which the Mn^{II} ion is located in an environment in which the zero-field splitting is in the range (*ca.* 10^{-2} to 10^{-1} cm^{-1}) between the Zeeman and the ^{55}Mn hyperfine energy.[18] This is apparent from the strong forbidden transitions between the allowed six-line pattern.

The superoxide dismutase activity of the complex was measured using the xanthine (3,7-dihydro-1*H*-purine-2,6-dione)–xanthine oxidase–nitro blue tetrazolium method.[19] The assay was performed in 50 mmol dm^{-3} potassium phosphate buffer (pH 7.4) at 25 °C in the absence of ethylenediaminetetraacetic acid. The results of the activity measurements are given in Fig. 2. The compound shows an IC_{50} value of 0.72 μmol dm^{-3} which indicates that it is a potent superoxide dismutase mimic. The IC_{50} value is the concentration of the complex which exerts the superoxide dismutase activity equal to one unit of the dismutase itself. The value reported here is one of the lowest for a manganese superoxide dismutase mimic and is close to the 0.75 μmol dm^{-3} reported by Nagano and co-workers[11] for a manganese(II) benzoate tris(pyrazolyl)borate complex. In both of these complexes manganese is in the divalent form. However, in dismutases containing manganese the metal is normally in the trivalent state, but can be reduced to the divalent state without any loss of enzymic activity.[20] It is of interest that the complex reported here and the tris(pyrazolyl)-borate complex reported earlier[11] have a N_3-co-ordination sphere and lower than O_h symmetry with azine and azol type of ligands. It is probable that the co-ordination of such nitrogen bases and their particular geometry leads to high superoxide dismutase activity. It is also worth noting that many of the high

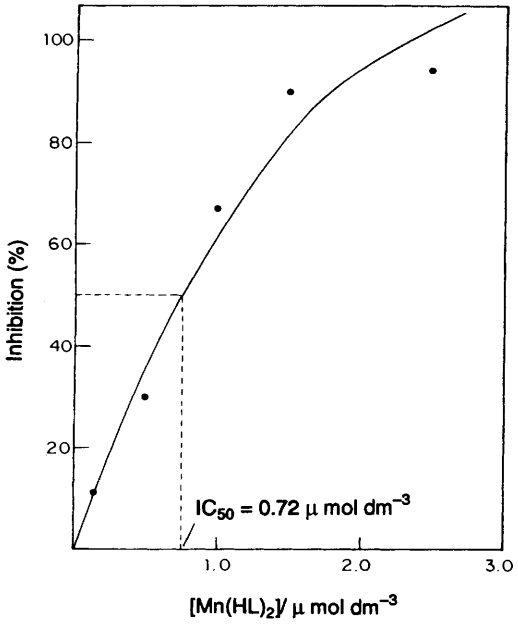


Fig. 2 Superoxide dismutase activity of $Mn(HL)_2$

activity complexes which include these two complexes and a manganese(III) bis(salicylidene)ethylenediamine complex[10d] are neutral rather than anionic. This may be an indication that maintaining the manganese in a neutral state leads to improved catalytic activity.

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