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Ligand Tuning of Single-Site Manganese-based Catalytic Antioxidants with Dual Superoxide Dismutase and Catalase Activity

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A bio-inspired manganese(II) complex with a linear pentadentate ligand framework containing soft sulfur donors and an alternating NSNSN binding motif displays excellent dual CAT/SOD-like antioxidant activity with high turnover efficiency and good operation stability in aqueous environment.

As a by-product of respiration, oxidative stress in living systems is a major source of irreversible cell damage and severe pathologies. Failure of the four electron O$_2$ reduction chain can give rise to an unbalanced build-up of so-called Reactive Oxygen Species (ROS), namely the superoxide radical (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$). These powerful oxidants can attack tissues, membranes and their proteic environment, thereby turning into lethal agents against cell structure and functioning. In-vivo protection occurs via suppression of the ROS cytotoxins through a cascade of dismutation processes. These reactions are mediated by two key classes of metallo-enzymes: superoxide dismutases (SODs) and catalases (CATs), both of which exist as manganese forms.

Mn(SOD)s are oxidoreductases with a mononuclear active site that cycles between the reduced Mn(II) and the oxidised Mn(III) state, whereas Mn(CAT)s contain a dinuclear Mn core with bridging carboxylate and oxide ligands (Scheme 1). A cooperative antioxidant effect against ROS injury stems from cascade reactions of SOD-mediated conversion of the superoxide radical to oxygen and hydrogen peroxide, followed by decomposition of hydrogen peroxide to water and oxygen by CAT.

Scheme 1. Cooperative SOD/CAT actions for ROS detoxification by manganese-based enzymes.

Recent efforts have been dedicated to the discovery of novel synthetic single-site Mn complexes with both SOD and CAT activity that can be used as artificial small molecule catalysts for ROS detoxification, following bio-inspired guidelines. Indeed, some Mn(III)-porphyrin and -salen complexes (for example AEOL10150 and EUK-134) as well as seven-coordinate Mn(II) complexes with macrocyclic aza-ligands (M40403) have entered clinical trials as synthetic catalytic antioxidants.

Figure 1: Seven-coordinate Mn(II) complexes containing linear pentadentate ligands with N, O and S donors (1-3) and the structures of the related SOD mimics M40403 and EUK-134. Molecular structure of A) [Mn(1)Cl$_2$] and B) [Mn(2)(OTf)$_2$] (50% probability ellipsoids).

We present here our studies on a series of seven-coordinate Mn(II) complexes with linear pentadentate ligands 1-3 (Figure 1) and their potential as catalytic antioxidants by evaluating their activity as CAT and SOD mimics. The series comprises isostructural ligands with both hard nitrogen and oxygen donors (NMe$_2$Py$_3$ (1), (NTos)$_2$Py$_3$ (1') and O$_2$Py$_3$ (2)) as well as a soft sulfur-containing analogue S$_2$Py$_3$ (3). In previous observations with
a pentadentate SNNNS manganese complex, sulfur donors were found to inhibit antioxidant activity.\textsuperscript{10} In contrast, we show herein that the [Mn(3)(OTf)\textsubscript{2}] complex with an alternating SNNSN binding motif, shows dual CAT and SOD activity \textit{both in CH\textsubscript{3}CN and in aqueous solution}, the highest in the series, surpassing the nitrogen and oxygen-based complexes.

Details regarding the synthesis and characterisation of the ligands and metal complexes are provided in the Supporting Information. A seven-coordinate geometry is confirmed for complexes [Mn(1)Cl\textsubscript{2}] and [Mn(2)(OTf)\textsubscript{2}] in the solid state (Figure 1) and which is comparable to the macrocyclic Mn(II) complex related to M40403\textsuperscript{11}. In all cases, a distorted pentagonal bipyramidal coordination geometry is observed with a displaced metal in acetonitrile or aqueous solutions to give complexes of the type [Mn(1)Cl\textsubscript{2}] and [Mn(2)(OTf)\textsubscript{2}], the metal lies ca. 0.09 and 0.08 Å out of the N(1) pyridyl ring plane, whereas in the M40403 relative, the metal lies ca. 0.45 Å out of this plane.

Triflate ligands are generally weakly coordinating and are easily displaced in acetonitrile or aqueous solutions to give complexes of the type [Mn(L)(S)\textsubscript{2}(OTf)\textsubscript{2}] with coordinated solvent ligands (S = acetonitrile or water) and uncoordinated triflate anions. The Mn(II) complexes studied here are high spin over the temperature range from 233 and 343 K in acetonitrile solution, with magnetic moment values of 5.9-6.0 \(\mu_B\) (see Figure S1). NMR spectroscopy is normally not useful for Mn(II) complexes due to the extreme line-broadening caused by the paramagnetic Mn(II) centre. However, the \(^{19}F\) NMR spectra for [Mn(1)(OTf)\textsubscript{2}] in CD\textsubscript{3}CN over the temperature range from 233 and 343 K do show a broad signal at approximately -70 ppm, which can be assigned to non-coordinating triflate anions and this supports that this complex exists as [Mn(1)(CD\textsubscript{3}CN)\textsubscript{2}] in CD\textsubscript{3}CN solution (see Figures S3 and S4). No geometrical changes in coordination, as seen in the case of the related iron complex [Fe(1)(OTf)\textsubscript{2}]\textsuperscript{12} were observed for any of these manganese complexes, possibly a consequence of the high spin d\(^{5}\) configuration and the absence of any ligand field stabilisation.

Cyclic voltammograms in acetonitrile are featureless between -1.5 V and +1.5 V (vs. Ag/AgNO\textsubscript{3}) (see Figure S2), as was noted for related dicationic seven-coordinate Mn(II) complexes.\textsuperscript{13, 14} All complexes are essentially colourless and the UV-Vis spectra are featureless at wavelengths above 350 nm. Charge transfer and d-d transitions are not expected in the visible region for these Mn(II) complexes and the activity appears to be strongly affected by the nature of the ligand, increasing in the order (NTos)\textsubscript{2}Py\textsubscript{3} (< S\textsubscript{2}Py\textsubscript{3} (< O2Py\textsubscript{3} (< O2Py\textsubscript{3} (Figure 2). The progressive addition of water to the acetonitrile solution resulted in a decrease in the activity of [Mn(3)(OTf)\textsubscript{2}] (Figure S8). As more water is produced during the dismutation reaction, this may explain the inhibition at longer reaction times seen in Figure 2. The change in solvent composition (acetonitrile/water) can have a significant effect on pK\textsubscript{a} values. Indeed, the pH of the solution appears to have a major influence on the stability of the catalyst system. The addition of small amounts of acid (HCl, 0.1M, 10 \(\mu\)L) resulted in an immediate loss of CAT activity, whereas the addition of base (NaOH, 1M, 10 \(\mu\)L) generated a very active (R\textsubscript{a} = 317 \(\mu\)M/min) and remarkably stable catalyst performance and resulted in quantitative O\textsubscript{2} evolution within 5 hours (entry 7 in Table 1 and Figure S9). Quantitative O\textsubscript{2} evolution was also achieved in \textit{aqueous solution}, using a borate buffer (pH 9) and the addition of imidazole.\textsuperscript{18, 19, 20} Under these conditions, a steady oxygen evolution was observed during 14h with quantitative conversion of H\textsubscript{2}O\textsubscript{2} (entry 8, Table 1 and Figure S10).

The novel seven-coordinate Mn(II) complexes of ligands 1-3 also show SOD-like activity in aqueous environment. The SOD...
reactivity of all complexes was evaluated using the NBT (nitro blue tetrazolium chloride) method, which allows monitoring of the catalytic removal of O₂ generated in a xanthine/xanthine oxidase system. SOD activities were determined as IC₅₀ (50% inhibition of NBT reduction) and compared on the basis of the kinetic constant (k₅₀EC) when available (Table 2 and SI). Overall, the Mn(II) complexes with ligands 1-3 show similar or lower activity compared to the reference complexes EUK-113 and the salen complexes. Noteworthy, the sulfur-containing complex [Mn(3)(OTf)₂] provides the highest SOD-like activity within the series with a similar rate constant compared to the benchmark catalyst M40403, which is about two orders of magnitude lower than the natural enzyme.

Conclusions

Mn(II) complexes containing linear pentadentate ligands with an alternating NXXNXN binding motif (X= N, O, S) are active single-site catalysts for the dismutation of superoxide and H₂O₂ to oxygen in acetonitrile and, more importantly, in aqueous environment. In particular the sulfur-containing complex [Mn(3)(OTf)₂] has been found to exhibit high dual CAT/SOD-like activity with excellent catalyst stability in the presence of added base. The sulfur donors in ligand 3 are believed to generate a strong ligand field resulting in increased complex stability under the oxidising testing conditions. Future studies will be directed to identify the key intermediates involved in these dismutation reactions and establish structure-reactivity relationships.

Notes and references

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c) Cytochrome 1.0 mM, 1200 units/mL catalase, 50 mM xanthine and sufficient xanthine oxidase to produce a rate of reduction of cytochrome cII of 0.025 absorbance unit (at 550 nm) per minute; b) Xanthine 50 µM, NBT 100 µM, Xanthine oxidase 0.005 U/ml in phosphate buffer 50 mM pH 7.4, catalyst 0.6-1.8 µM. c) Various concentrations: 0.6-1.8 and 6 µM.


15. A caveat needs to be added as activities determined for different catalyst systems by different groups of researchers in different labs are not always strictly comparable.


18. In phosphate buffer (pH 9), the catalyst displays sluggish oxygen evolution activity, levelling off at 3% at a slower rate (R₂=18 µM/min), which may hint at a possible interaction between the borate buffer and the oxidant (see also Deary et al., Org. Biomol. Chem., 2013, 11, 309-317).

19. The exact role of imidazole is unclear at this stage, but the addition of imidazole has no effect in acetonitrile. In the absence of imidazole in aqueous solution, no oxygen evolution is observed using [Mn(3)(OTf)₂] and H₂O₂ in borate buffer at pH 9.2. Furthermore, no oxygen evolution was observed using MnCl₃·H₂O, imidazole, H₂O₂, and borate buffer at pH 9.2, but oxygen evolution commenced upon addition of [Mn(3)(OTf)₂].

