Chelate electronic properties control the redox behaviour and superoxide reactivity of seven-coordinate manganese(II) complexes[†]

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We have synthesized and characterized two Mn(II) sevencoordinate complexes with N5 pentadentate ligands, which contain hydrazone and hydrazide groups respectively. We have shown that insertion of hydrazido (amido) groups into the ligand sphere increases the negative charge of the chelate, without changing a donor atom set and coordination geometry, and radically modulate a redox activity of sevencoordinate manganese complexes, which is important for the function of manganese as a superoxide dismutase catalytic center.

Superoxide (O_2^{-}) reactions with metal centers are of interest since they occur within enzymatic catalysis (superoxide dismutases, SOD, and superoxide reductases, SOR)^{1,2} as well as in undesired processes that might cause pathophysiological conditions. Their understanding helps in conceiving whether and how metal complexes can be used as pharmaceuticals for treatment of disease states caused by O₂⁻ overproduction and possible negative effects of superoxide interactions with metal centers under physiological conditions. Up to now the most active SOD mimetics are sevencoordinate macrocyclic Mn(II) complexes of pentaaza crowns which have been considered as clinical candidates for a variety of inflammatory conditions.^{3,4} The effects of chelate substituents on the SOD activity of that class of complexes have been extensively investigated, and it has been concluded that their prominent conformational flexibility is the key property that assists the high SOD activity.5

We have recently shown that an acyclic and rigid pentadentate chelate system (H₂dapsox = 2,6-diacetylpyridinebis(semioxamazide)) can also be considered as structural motif that supports the SOD-activity of iron and manganese complexes.⁶ The advantage of such types of complexes is that their syntheses are much easier and with higher yields than the syntheses of the corresponding macrocyclic ones. To understand the effects of electronic properties of acyclic and more rigid pentadentate chelates on redox and structural features of the corresponding Mn(II) complexes and their reactivity towards O₂⁻, we have now synthesized and characterized the seven-coordinate complexes [Mn^{II}(Dcphp)(CH₃OH)₂](CH₃OH)₂ (1) (H₂Dcphp = N'^2 , N'^6 -di(pyridin-2-yl)pyridine-2,6-dicarbohydrazide) and $[Mn^{II}(H_2Daphp)(H_2O)_2](ClO_4)_2$ (2) $(H_2Daphp = 2,6-bis((2-(pyridin-2-yl)hydrazono)ethyl)pyridine),‡ and studied their reaction with KO₂ electrochemically, spectrophotometrically and by sub-millisecond mixing UV/vis stopped-flow in DMSO, in a manner recently reported by us.⁶$

X-Ray structure determinations§ (Tables S1 and S2)† of both complexes show (Fig. 1 and Fig. 2) a distorted pentagonalbipyramidal geometry around Mn^{II} with five nitrogen atoms of the pentadentate chelates in the equatorial positions and two oxygen atoms of methanol or water as axial donors. Both the bisdeprotonated Dcphp²⁻ hydrazide, as well as the neutral H₂Daphp hydrazone, coordinate to Mn(II) in a kind of helical mode, with a dihedral angle between the disadjacent five-membered rings



Fig. 1 Thermal ellipsoid representation of $[Mn^{II}(Dcphp)(CH_3OH)_2)]$ in the crystal structure of 1 (50% probability level).



Fig. 2 Thermal ellipsoid representation of $[Mn^{II}(H_2Daphp)(H_2O)_2]^{2+}$ in the crystal structure of 2 (50% probability level).

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[N5-N6-C13-N7-Mn1] and [N2-N3-C7-N4-Mn1] of 22.7° in 1, and a corresponding dihedral angle in 2 of 24.8°. Compared to the structure of the free H₂Dcphp ligand (Fig. S1 and Table S3),[†] the geometry of the hydrazide groups in 1 changes substantially upon coordination. The average Chydrazide-N bond distance decreases from 1.34 to 1.31 Å and the average C_{hvdrazide}-O distance increases from 1.23 to 1.29 Å. The average Mn1–N_{hydrazide} bond length (2.267 Å) is significantly shorter than the average $Mn1\text{--}N_{\text{pyridine}}$ bond length (2.386 Å) (Table S2).† These structural features result from the chelate's negative charge and its coordination in a hybrid form of the deprotonated hydrazide-HN-N-C=O and α -oxiazine-HN-N=C-O⁻ resonance structures. The asymmetry in the two Mn-N_{terminal pyridine} bonds in the crystal structure of 1 is prominent (Mn1–N4 = 2.467(2) Å vs. Mn1–N7 = 2.360(2) Å), which is not typical for high-spin d^5 electronic configurations. The intra- and especially intermolecular hydrogen bonds (N6-H \cdots O) within the crystal packing are responsible for this asymmetry, similar to that observed in the structure of [Mn^{II}(H₂dapsox)(CH₃OH)(H₂O)]²⁺.⁶ The lack of the carbonyl groups in H₂Daphp makes it less acidic, resulting in its coordination in the neutral hydrazone form, which is reflected in a longer average Mn1-N_{imine} bond length and general higher symmetry in the Mn-N bond distances in the structure of 2 compared to those in 1.

The cyclic voltammogram of 1 in DMSO purged with nitrogen exhibits a quasi-reversible Mn^{III}/Mn^{II} couple at 0.372 V and irreversible reduction process at -0.325 V vs. Ag/AgCl (Fig. 3), which is confirmed to be ligand centered by measuring the cyclic voltammogram of free H₂Dcphp under the same conditions. However, under the same experimental conditions 2 is electrochemically silent in the scan range from -1 to 1.2 V. These results demonstrate the effect of the deprotonated amide (hydrazide) groups and a consequent increase of the ligand negative charge on the redox behaviour of the manganese center. A strong σ -donor ability of the negatively charged hydrazido nitrogen in the coordination sphere of manganese significantly decreases the Mn^{III}/Mn^{II} redox potential by stabilizing the Mn^{III} form of the complex, which was characterized by spectro-electrochemical measurements (λ_{max} = 360-390 nm, inset in Fig. 3a). Alternating bulk electrolyses at 0.5 V (inset of Fig. 3a) and 0 V vs. Ag/AgCl (Fig. S2)[†] show that 1 can be oxidized and reduced in a quantitatively reversible manner. In iron chemistry it is known that one amido nitrogen as a donor atom within TPA (tris-(2-pyridylmethyl)amine) type chelates stabilizes the Fe³⁺ oxidation state in the six-coordinate geometry by about 1 V.7 However, in the seven-coordinate geometry, two deprotonated amide groups of the macrocyclic ligand stabilize the Fe³⁺ oxidation state only by about 0.3 V.⁸ In the case of manganese complexes it is known that the tetraamido and diamidodialkoxido ligands stabilize high-valent Mn=O species.9,10 However the effect of the amide group on the redox behaviour of seven-coordinate manganese complexes has not been reported. It has been qualitatively demonstrated that the bound carboxamido nitrogen to the six-coordinate Mn center makes the complex more sensitive to oxidation in comparison to the analogous Schiff base complex.¹¹ Our results show that in the seven-coordinate geometry, coordination of two hydrazido nitrogens instead of two imine nitrogens stabilizes the Mn^{III} oxidation state by at least 0.8 V.

Cyclic voltammetric measurements⁶ were also performed in oxygen saturated DMSO ($[O_2] = 2.1 \text{ mM}$) in the scan range



Fig. 3 Cyclic voltammograms of: a) 1 purged with nitrogen (inset: spectro-electrochemical oxidation of 1 at 0.5 V vs. Ag/AgCl); b) 1 purged with nitrogen (A), oxygen (B), and pure DMSO purged with oxygen (C). Conditions: $[1] = 0.5 \times 10^{-3}$ M, $[Bu_4NPF_6] = 0.1$ M, T = 298 K, scan rate = 0.5 V s⁻¹.

from 1 to -1.5 V (Fig. 3b), and when the scan proceeds toward more negative potentials, superoxide is generated by reduction of oxygen. When the scan is returned toward positive potentials, no anodic peak assigned to the oxidation of O_2^- is found, suggesting that **1** present in solution (experiments were performed with [**1**] = 1 mM and 0.1 mM) decomposes superoxide. The same experiment was performed in the presence of free ligand and the disappearance of the anodic peak assigned to the oxidation of O_2^- was not observed. Consequently, decomposition of O_2^- in the presence of **1** is related to the metal-centered redox process. Complex **2** does not affect the superoxide reoxidation in the same type of experiments.

The reaction of **1** with a large excess of KO₂ in DMSO containing a controlled amount of water (0.06%) was followed by time resolved UV/vis spectroscopy, and the rapid decomposition of O_2^- was quantified by following the corresponding absorbance decrease at 270 nm (best fitted as a first-order process) in a series of a sub-millisecond mixing stopped-flow measurements, in which the catalytic concentration of the studied complexes was varied.⁶

A good linear correlation between the corresponding k_{obs} value and the complex concentration was observed, and from the slope of the plot the catalytic rate constant (k_{cat}) was determined to be (6.1 ± 0.7) × 10⁶ M⁻¹ s⁻¹ (Fig. 4). The products of superoxide disproportionation, O₂ and H₂O₂, were qualitatively



Fig. 4 Plot of k_{obs} for the decay at 270 nm *versus* [1]. Conditions: $[O_2^{-1}] = 2 \text{ mM}$, 25 °C in DMSO. Inset: UV/vis spectra recorded for the reaction of 5×10^{-5} M of 1 with 1 mM KO₂ in DMSO at room temperature. A: spectrum recorded before mixing; B: final spectrum obtained after decomposition of KO₂.

detected.⁶ Upon superoxide decomposition the complex remains in solution in the Mn^{III} form ($\lambda_{max} = 360-390$ nm, inset in Fig. 3), similar to what was observed in the case of the SOD active [Mn^{II}(H₂dapsox)(CH₃OH)(H₂O)]²⁺ complex.⁶ As expected from the electrochemical behaviour of **2**, its mixing with an excess of KO₂ in DMSO does not cause rapid decay of the absorbance characteristic for superoxide.¹²

The presented results reveal that 1 catalyzes the fast disproportionation of superoxide under the applied experimental conditions, whereas 2 does not show any SOD activity. This can be explained by the high redox potential of 2, which does not fall between the redox potentials for the reduction and oxidation of O_2^- under the applied experimental conditions. However, for the Mn complexes with redox potentials within that window a correlation between their $E_{1/2}$ and SOD activities (see Table 1 for the seven-coordinate Mn complexes) is more complex. It seems that for the complexes with lower $E_{1/2}$, reduction of the Mn^{III} form is the rate-limiting step in the catalysis, whereas for the SOD mimetics with higher $E_{1/2}$, the catalytic cycle is a Mn^{II} oxidation limited process. Data reported in the literature for the SOD active Mn^{III} porphyrins¹³ and Mn complexes based on N-centered ligands¹⁴ also follow such a trend.

To probe whether the ability of **1** and **2** to form six-coordinate complexes in their Mn^{II} and Mn^{III} oxidation states upon release of a coordinated solvent molecule, has an influence on their reactivity towards O_2^- in a manner that was reported for the macrocyclic Mn(II) SOD mimetics,^{15,16} we performed DFT calculations.¹⁷ The energies required for solvent dissociation¹⁸ were calculated to be +2.9 and -1.1 kcal mol⁻¹ for the Mn^{II} and Mn^{III} forms of **1**, respectively (Fig. S3).[†] These small energy differences between seven- and six-coordinate geometries of both oxidation states, suggest that solvent dissociation and formation of a six-coordinate

 Table 1
 $E_{1/2}$ and SOD activities of the seven-coordinate Mn complexes

Complex	1	Mn ^{II} (H ₂ dapsox) ^a	Mn ^{II} (pyaneN5) ^a
$\frac{1}{E_{1/2}/V}$ vs. Ag/AgCl k_{cat}/M^{-1} s ⁻¹	$\begin{array}{c} 0.37 \\ (6.1 \pm 0.7) \times 10^6 \end{array}$	0.66 $(1.2 \pm 0.3) \times 10^7$	$0.80 \\ (5.3 \pm 0.8) \times 10^{6}$
a Obtained under the same experimental conditions as in the present work. 6			

intermediate are not crucial for the SOD activity of the complex, in agreement with our recent findings.^{6,19} In the case of **2**, the solvent dissociation energies for its Mn^{II} and Mn^{III} species are +1.7 and +7.9 kcal mol⁻¹, respectively (Fig. S4).† The latter value indicates that formation of the six-coordinate Mn^{III} form of **2** is significantly less favorable than in the case of **1**. This structural feature together with the electronic properties of H₂daphp might contribute to the low stability of the Mn^{III} oxidation state of **2** and consequently its high $E_{1/2}$. Interestingly, in the case of macrocyclic Mn(II) SOD mimetics, ligand structural features do not affect their redox potential, but have an influence on their SOD activity.⁵

In conclusion, we have shown how insertion of amido groups into the ligand sphere, without changing the donor atom set and coordination geometry, can radically modulate the redox activity of seven-coordinate manganese complexes, which is important for the function of Mn as catalytic center.²⁰ It still remains to be seen to what extent the ligand structural and electronic features can control the SOD activity of these complexes by affecting the mechanism (associative *vs.* dissociative) of their substitution processes. Studies along these lines are in progress.

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Notes and references

‡ Preparation of H₂Dcphp: to a solution of 2-hydrazinopyridine (403 mg, 3.70 mmol) in 10 mL of absolute dichloromethane under nitrogen, degassed triethylamine (0.410 mL, 2.96 mmol) was added. After that a solution of 2,6-pyridinedicarbonyl dichloride (302 mg, 1.48 mmol) in 30 mL of dichloromethane was added. After 2 h of stirring, the solvent was evaporated and the residue was washed several times with a saturated solution of NaHCO₃ and with water to give H₂Dcphp (321 mg, 62%) as a white solid. $\delta_{H}(300 \text{ MHz}, \text{DMSO-d}_{6}, 25 \,^{\circ}\text{C})$ 11.2 (s, 2H, CONH-NH); 8.20 (m, 3H, Pyridyl- $H_{(CON)2}$); 8.07 (d, 2H, Pyridyl(6) H_{NH}); 7.54 (m, 2H, Pyridyl(4) H_{NH}); 6.72 (m, 2H, Pyridyl(5) H_{NH}); 6.68 (d, 2H, Pyridyl(3) H_{NH}); $\delta_{c}(100 \text{ MHz}, \text{DMSO-d}_{6}, 25 \,^{\circ}\text{C})$ 162.7, 159.6, 148.2, 147.6, 139.7, 137.5, 124.8, 114.7, 106.7; Anal. calcd. for C₁₇H₁₅N₇O₂·H₂O: C, 55.58; H, 4.66; N, 26.69%. Found: C, 56.04; H, 4.52; N, 26.94%.

Synthesis of complex 1: to a stirred suspension of H₂Dcphp (0.367 g, *ca.* 1 mmol) in absolute methanol (50 mL), a methanol solution (10 mL) of MnCl₂·2H₂O (0.852 g, 1 mmol) was added. After 1 h of stirring, NaOCH₃ (0.108 g, 2 mmol) was carefully added to the solution under nitrogen atmosphere. After two hours of refluxing, the red solution was filtered and concentrated to *ca.* 30 mL and 0.5 g of NaClO₄ was added. The solution was kept in a refrigerator overnight. Shiny deep-yellow crystals of **1** (0.14 g, 26%) were collected and washed with small amounts of acetone. v_{max} (KBr)/cm⁻¹ 3329 s(NH), 3066 s(H₂O), 2363 m, 1701 s, 1613 s, 1516 m(amide C=O), 1341 s (amide), 1191 m (CH), 999 m, 692 m; Anal. calcd. for C₂₁H₂N₇O₆Mn: C, 47.55; H, 5.51; N, 18.48%. Found: C, 47.04; H, 5.12; N, 18.49%.

Synthesis of complex **2**: 2,6-diacetylpyridine (1.63 g, 10 mmol) and 2-hydrazinopyridine (2.18 g, 20 mmol) were added to 50 mL of methanol and the mixture was stirred at 55 °C for 1 h. A solution of Mn(ClO₄)₂·4H₂O (3.26 g, 10 mmol) in 20 mL methanol was added dropwise to the resulting white suspension. The solution color changed to yellow, while some of the white powder was still undissolved. The addition of 30 mL of CH₃CN resulted in a clear yellow solution. After 3 h of refluxing the hot reaction mixture was filtered and the dark yellow residue discarded. The deep orange solution was then allowed to cool to room temperature and kept in a refrigerator, rendering deep orange crystals of **2** (3.67 g, 57.8%). v_{max} (KBr)/cm⁻¹ 3290 s(NH), 3198 s(H₂O), 3112 s, 2362 m, 1701 w, 1687 s, 1551 m, 1341 m(amide), 1279 m, 1243 m(CH) 1085 w, 819 m, 775 m,

630 m(py); Anal. calcd. for $C_{19}H_{23}Cl_2N_7O_{10}Mn:$ C, 35.92, H, 3.65, N, 15.43%. Found: C, 35.47, H, 3.64, N 15.71%.

§ Crystal data for 1: C₂₁H₂₉N₇O₆Mn, FW = 530.45, monoclinic, space group C2/c, a = 25.251(2) Å, b = 7.6250(5) Å, c = 27.574(2) Å, $\beta = 113.044(5)^{\circ}$, V = 4885.4(6) Å³, Z = 8, T = 100(2) K, $D_c = 1.442$ g cm⁻³; 55979 reflections collected ($R_{int} = 0.0550$), 5386 unique reflections, $R_1 = 0.0396$ [$I > 2\sigma(I)$], w $R_2 = 0.0882$ (all data), GOF = 1.129. CCDC 720837. For 2: C₁₉H₂₃Cl₂N₇O₁₀Mn, FW = 635.28, monoclinic, space group C2/c, a = 10.9947(6) Å, b = 31.380(4) Å, c = 7.9492(6) Å, $\beta = 108.613(5)^{\circ}$, V = 2599.1(4) Å³, Z = 4, T = 100(2) K, $D_c = 1.623$ g cm⁻³; 31490 reflections collected ($R_{int} = 0.0653$), 3105 unique reflections, $R_1 = 0.0331$ [$I > 2\sigma(I)$], w $R_2 = 0.0754$ (all data), GOF = 1.052. CCDC 720838. For H₂Dcphp: CCDC 720839.

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