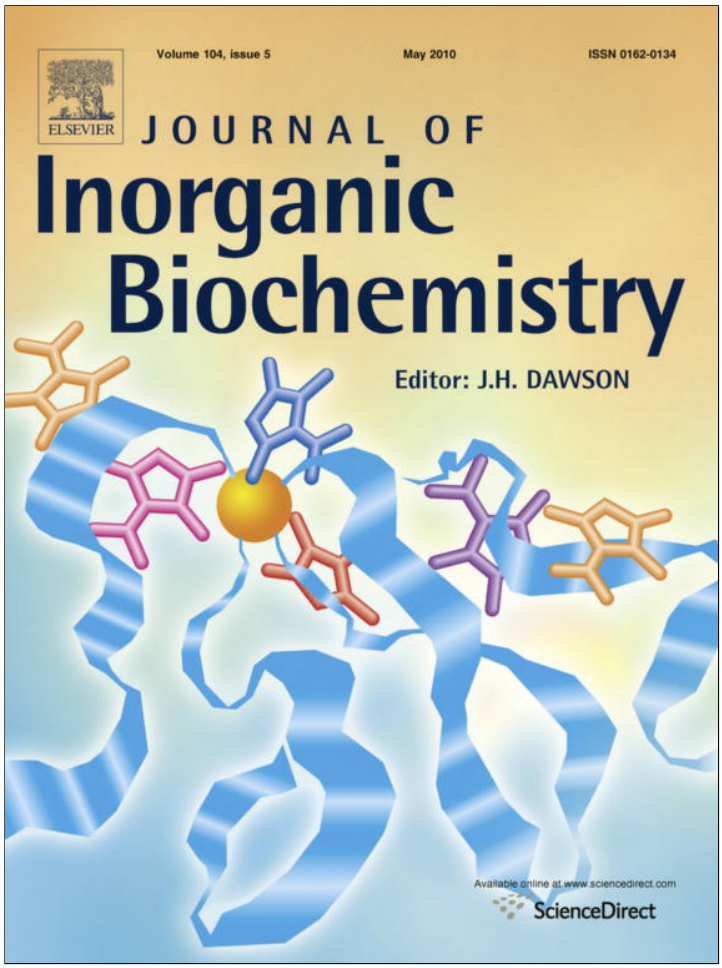


Journal of Inorganic Biochemistry 104 (2010) 496–502

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Synthesis, characterization and antioxidant activity of water soluble Mn^{III} complexes of sulphonato-substituted Schiff base ligands

Diego Moreno [a], Verónica Daier [a], Claudia Palopoli [a], Jean-Pierre Tuchagues [b,c], Sandra Signorella [a,*]

[a] IQUIR (Instituto de Química Rosario) – CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina
[b] CNRS, LCC (Laboratoire de Chimie de Coordination), 205, route de Narbonne, F-31077 Toulouse, France
[c] Université de Toulouse, UPS, INPT, LCC, F-31077 Toulouse, France

ARTICLE INFO

Article history:
Received 30 September 2009
Received in revised form 17 December 2009
Accepted 18 December 2009
Available online 6 January 2010

Keywords:
Water soluble Mn complexes
Schiff base ligands
SOD models
Catalase activity

ABSTRACT

Two new Mn^{III} complexes Na[Mn(5-SO_3-salpnOH)(H_2O)] · $5H_2O$ (1) and Na[Mn(5-SO_3-salpn)(MeOH)] · $4H_2O$ (2) (5-SO_3-salpnOH = 1,3-bis(5-sulphonatosalicylidenamino)propan-2-ol, 5-SO_3-salpn = 1,3-bis(5-sulphonatosalicylidenamino)propane) have been prepared and characterized. Electrospray ionization-mass spectrometry, UV–visible and ^{1}H NMR spectroscopic studies showed that the two complexes exist in solution as monoanions [Mn(5-SO_3-salpn(OH))(solvent)$_2$]$^-$, with the ligand bound to Mn^{III} through the two phenolato-*O* and two imino-*N* atoms located in the equatorial plane. The $E_{1/2}$ of the Mn^{III}/Mn^{II} couple (−47.11 (1) and −77.80 mV (2) vs. Ag/AgCl) allows these complexes to efficiently catalyze the dismutation of $O_2^{\cdot-}$, with catalytic rate constants 2.4×10^6 (1) and 3.6×10^6 (2) M^{-1} s^{-1}, and IC_{50} values of 1.14 (1) and 0.77 (2) μM, obtained through the nitro blue tetrazolium photoreduction inhibition superoxide dismutase assay, in aqueous solution of pH 7.8. The two complexes are also able to disproportionate up to 250 equivalents of H_2O_2 in aqueous solution of pH 8.0, with initial turnover rates of 178 (1) and 25.2 (2) mM H_2O_2 min^{-1} mM^{-1} $catalyst^{-1}$. Their dual superoxide dismutase/catalase activity renders these compounds particularly attractive as catalytic antioxidants.

1. Introduction

Superoxide dismutases (SODs) protect biological systems against oxidative damage caused by the superoxide radical ($O_2^{\cdot-}$) generated during aerobic metabolism through monoelectronic reduction of molecular oxygen [1]. High levels of superoxide are associated to several pathologies like diabetes, oxidative damage, numerous neurodegenerative disorders such Alzheimer's and Parkinson's diseases [2–5], and some types of cancer originating from DNA mutations induced by superoxide [6,7]. MnSOD is found in mitochondria and chloroplasts of eukaryotes and in the cytoplasm of bacteria [8,9]. Crystal structures have been solved for MnSOD from both bacteria (*Thermus thermophilus* [10] and *Escherichia coli* [11]) and eukaryotes (human mitochondria [12]). The active site of MnSOD contains one Mn ion in a distorted trigonal–bipyramidal N_3O_2 environment, coordinated by three histidine residues, one aspartate and one OH^- ion/H_2O molecule (Fig. 1) [11,13]. These enzymes catalyze the dismutation of $O_2^{\cdot-}$ into H_2O_2 and O_2 through a "ping-pong" mechanism between Mn^{II} and Mn^{III} oxidation states [14].

The use of exogenous SODs (e.g. bovine) as therapeutic agents for treatment of a variety of disorders in which superoxide has a significant role has been tested. However, it is difficult to employ SODs in vivo, because of their immunogenic response [15] and high molecular mass (≈30 kDa) that disables the enzyme to cross the cell membranes [16]. The difficulties associated with the use of exogenous SOD have stimulated the search of low molecular weight catalytic scavengers as a new generation of pharmaceutical compounds. Many complexes of Mn have been reported to be capable of functioning as catalysts for the dismutation of superoxide and have been examined as catalytic antioxidants in preclinical trials, the most efficient being Mn^{III}-salen (salen = 1,2-bis(salicylidenamino)ethane) [17], Mn^{III}-porphyrinato [18–21] and Mn^{II}-1,4,7,10,13-pentaazacyclopentadecane [22–24]. Among them, Mn^{III}-salen complexes have been reported to have dual SOD/CAT (catalase) activity, an advantageous property since SOD activity alone would produce cytotoxic H_2O_2. However, the tested salen complexes are only slightly soluble in water and loose activity in a few minutes under the conditions of the catalase assay [25]. Therefore, there is a need to improve the stability of this class of complexes under physiological conditions to be useful as drugs.

Herein, we report the synthesis, characterization, properties and SOD/CAT activity of two novel water soluble manganese complexes Na[Mn(5-SO_3-salpnOH)(H_2O)] (1) and Na[Mn(5-SO_3-salpn)(MeOH)] (2) obtained with the sodium salts of the Schiff base ligands 1,3-bis(5-sulphonatosalicylidenamino)propan-2-ol (5-

* Corresponding author. Tel.: +54 341 4350214; fax: +54 341 4370477.
E-mail address: signorel@infovia.com.ar (S. Signorella).

0162-0134/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2009.12.016

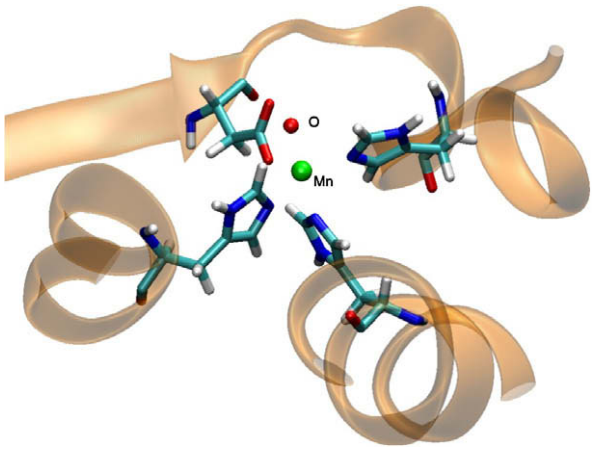
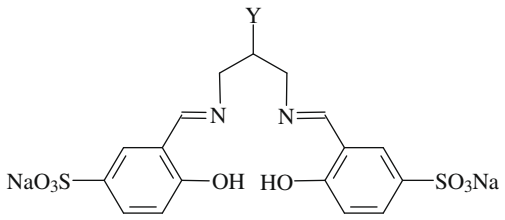


Fig. 1. Active-site structure of human mitochondrial MnSOD based on coordinates from the PDB file 1N0J [12].



Scheme 1. Disodium salts of 5-SO_3-salpnOH (Y = OH) and 5-SO_3-salpn (Y = H) used in the synthesis of the complexes.

SO_3-salpnOH) and 1,3-bis(5-sulphonatosalicylidenamino)propane (5-SO_3-salpn) (see Scheme 1), and compare their SOD activity with that of the complex obtained with 1,2-bis(5-sulphonatosalicylidenamino)ethane (5-SO_3-salen).

2. Experimental section

2.1. Materials

All reagents or analytical grade chemicals were used as purchased. Solvents were purified by standard methods. The concentration of H_2O_2 stock solution was determined by iodometric titration. Sodium salicylaldehyde-5-sulphonate was prepared according to a published method [26,27].

2.2. Physical measurements

Electronic spectra were recorded on a JASCO V550 spectrophotometer with thermostated cell compartments. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrophotometer. ESI-mass spectra were recorded on a Perkin-Elmer SCIEX 365 LCMSMS mass spectrometer. The electrospray solutions were prepared from water solutions of the complexes and diluted with methanol to $a \approx 10^{-5}$ M concentration at a flow rate of 5 μL min^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 200 NMR spectrometer at ambient probe temperature (ca 26 °C), with nominal operating frequencies of 200.1 and 50.3 MHz. Variable-temperature magnetic susceptibility data were obtained with a Quantum Design MPMS SQUID susceptometer, under a magnetic field of 0.5 T in the temperature range 2–300 K. Diamagnetic corrections were applied by using Pascal's constants [28]. Least-squares fittings were accomplished with an adapted version of the function-minimization program MINUIT [29]. Conductivity measurements were performed using a Horiba F-54 BW conductivity meter, on 1.0 mM solutions of the complexes in water. The electrochemical experiments were performed with a computer-controlled Princeton Applied Research potentiostat, model VERSASTAT II, with model 270/250 Research Electrochemistry Software. Studies were carried out under Ar, in water solutions using 0.1 M KNO_3 as supporting electrolyte and $\sim 10^{-3}$ M of the complex. The working electrode was a Pt wire and the reference electrode was Ag/AgCl with Pt as the auxiliary electrode.

2.3. Synthesis of ligands

2.3.1. Disodium salt of 1,3-bis(5-sulphonatosalicylidenamino)propan-2-ol (5-SO_3-salpnOH)

Na_2[5-SO_3-salpnOH] was prepared by Schiff-base condensation of sodium salicylaldehyde-5-sulphonate (200 mg, 0.825 mmol) with 1,3-diaminopropan-2-ol (37 mg, 0.413 mmol) in ethanol (40 mL), at reflux for 6 h. Na_2[5-SO_3-salpnOH] was isolated as a pure yellow solid by precipitation from the reaction mixture at room temperature. Yield: 171 mg (0.375 mmol, 91%). ^1H NMR (D_2O) δ: 8.35 (singlet (s), 2H, N=CH—), 6.5–7.8 (multiplet (m), 6H, Ar), 4.4–4.8 (m, 5H, N—CH_2—R and R_2—CH—OH). ^{13}C RMN (D_2O) δ: 175.52 (Ar), 168.62 (CH=N), 133.62, 132.48, 128.96, 122.41, 114.18 (Ar), 67.9 (R_2CH—OH), 54,90 (N—CH_2—CHOH—). Significant IR bands (KBr, ν cm^{-1}): ν_{OH} 3420 (broad), $\nu_{C=N}$ 1613, ν_{SO3} 1108/1034. UV–visible (UV–vis) λ_{max} (nm) in H_2O: 222, 234, 255 (sh), 330 (sh), 375.

2.3.2. Disodium salt of 1,3-bis(5-sulphonatosalicylidenamino)propane (5-SO_3-salpn)

Na_2[5-SO_3-salpn] was obtained from a mixture of sodium salicylaldehyde-5-sulphonate (500 mg, 2.57 mmol) and 1,3-diaminopropane (0.107 mL, 1.28 mmol) in 50 mL EtOH, stirred under reflux for 24 h. After cooling, the Schiff base precipitated from the reaction mixture as a pure yellow powder. Yield: 500 mg (1.14 mmol, 89%). ^1H NMR (D_2O) δ: 8.40 (s, 2H, N=CH—), 6.6–7.9 (m, 6H, Ar), 3.77 (triplet (t), 4H, N—CH_2—R), 2.11 (m, 2H, N—CH_2—CH_2—CH_2—N). ^{13}C NMR (D_2O, δ) 176.45 (Ar), 167.92 (CH=N), 133.53, 132.56, 128.46, 122.70, 113.86 (Ar), 49.00 (N—CH_2—R), 29.00 (N—CH_2—CH_2—CH_2—N). Significant IR bands (KBr, ν cm^{-1}): ν_{OH} 3405 (broad), $\nu_{C=N}$ 1612, ν_{SO3} 1108/1035. UV–vis λ_{max} (nm) in H_2O: 222, 236, 254 (sh), 331 (sh), 373.

2.4. Synthesis of complexes

2.4.1. Na[Mn(5-SO_3-salpnOH)(H_2O)] · 5H_2O (1 · 5H_2O)

$Mn(OAc)_2 \cdot 4H_2O$ (294 mg, 1.2 mmol) was added to a solution of Na_2[5-SO_3-salpnOH] (300 mg, 0.6 mmol) in methanol (12 mL) and left to stir for 1 h. The formed green precipitate was filtered off, washed with ether and dried under vacuum. Yield: 305 mg (0.48 mmol, 80%). Anal. calcd. for $MnC_{17}H_{16}N_2O_{10}S_2Na \cdot 5H_2O$: C 31.88, H 4.09, N 4.37, Mn 8.59, Na 3.58%; found: C 31.80, H 3.10, N 3.83, Mn 8.79, Na 4.18%. Although the analysis results for H and Na are somewhat unsatisfactory, the chemical formula is consistent with the NMR, IR and thermogravimmetric data. Significant IR bands (KBr, ν cm^{-1}): ν_{OH} 3478 (broad), ν_{CH} 2950, $\nu_{C=N}$ 1611, ν_{SO3} 1112/1029. UV–vis λ_{max} nm (ε $M^{-1}cm^{-1}$) in H_2O: 277 (23970), 363 (8980). Molar conductivity = 84 Ω^{-1} cm^2 mol^{-1}. The content of five molecules of non-coordinated water per complex molecule was confirmed by thermogravimetric analysis of the complex which showed 14% mass loss below 120 °C.

2.4.2. Na[Mn(5-SO_3-salpn)(MeOH)] · 4H_2O (2 · 4H_2O)

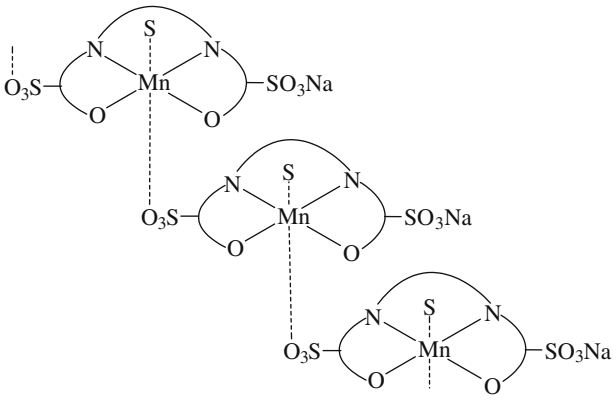
Na_2[5-SO_3-salpn] (450 mg, 1.01 mmol) and $Mn(OAc)_2 \cdot 4H_2O$ (373 mg, 1.5 mmol) were mixed in methanol (20 mL). A green powder formed immediately. The mixture was stirred for 1 h and the green solid was collected by filtration, washed with ether and dried under vacuum. Yield: 444 mg (0.72 mmol, 71%). Anal. Calcd. for $MnC_{18}H_{18}N_2O_9S_2Na \cdot 4H_2O$: C 34.84, H 4.22, N 4.51, Mn 8.85, Na 3.70%; found: C 35.02, H 3.60, N 5.08, Mn 8.92, Na 3.52%. Significant IR bands (KBr, ν cm^{-1}): ν_{OH} 3474(broad), ν_{CH} 2950, $\nu_{C=N}$ 1613, ν_{SO3} 1112/1029. UV–vis λ_{max} nm (ε $M^{-1}cm^{-1}$) H_2O: 278 (19060), 362 (6870). Molar conductivity = 111 Ω^{-1} cm^2 mol^{-1}. The content of four molecules of non-coordinated water per complex molecule was confirmed by thermogravimetric analysis of the complex, which showed 11.6% mass loss below 115 °C.

2.5. Indirect SOD assay

The SOD activity of the complexes was assayed by measuring inhibition of the photoreduction of nitro blue tetrazolium (NBT), by a method slightly modified from that originally described by Beauchamps and Fridovich [30]. The solutions containing riboflavin (3.4×10^{-6} M), methionine (0.01 M), NBT (4.6×10^{-5} M) and complex of various concentrations were prepared with phosphate buffer (pH 7.8). The mixtures were illuminated by a fluorescent lamp with a constant light intensity at 25 °C. The reduction of NBT was monitored at 560 nm with various illumination periods (t). Rates in the absence and in the presence of different concentrations of complex were determined and plotted vs. complex concentration. Inhibition percentage was calculated according to: $\{(\Delta Abs/t)_{without\ complex} - (\Delta Abs/t)_{with\ complex}\} \times 100/(\Delta Abs/t)_{without\ complex}$. The IC_{50} value represents the concentration of the SOD mimic that induces a 50% inhibition of the reduction of NBT. Control experiments were performed on mixtures of NBT + complex, riboflavin + complex, and NBT + methionine + complex, in phosphate buffer, to ensure that the complex does not react independently with any of the components of the mixture. Based on these experiments, cross reactivity of the complex with NBT or riboflavin was disregarded. Under conditions used in this work, the catalytic rate constant (k_{McCF}) for MnSOD was found to be 5.5×10^8 M^{-1} s^{-1}.

2.6. Disproportionation of H_2O_2

The H_2O_2 disproportionation catalyzed by 1–2 was measured by volumetric determination of the evolved O_2 from reaction mixtures in water, as previously described [31].



Scheme 2. One-dimensional {Na[Mn^{III}-5-SO_3-salpn(OH)]}$_n$ polymer in the solid state. S=H_2O (1) or MeOH (2).

3. Results and discussion

3.1. Synthesis of the complexes

Complexes **1** and **2** were prepared from 1:1 mixtures of the ligand with $Mn(OAc)_2$ in methanol. When $Mn(ClO_4)_2$ was used instead of acetate, no color change was observed until NaOH or Et_3N was added to the solution of complex, such as observed for previously reported Mn complexes of tetradentate Schiff base ligands [32–34]. Therefore, acetate facilitates the aerobic oxidation of Mn^{II} and deprotonation of the phenol for coordination to the metal. Although the disodium salts of the ligands were used, the analytical results show that the complexes retain only one sodium ion per complex molecule in the solid state, and the thermogravimmetric analyses indicate that only one of the solvent molecules is coordinated to the metal centre of each complex. The remaining coordination position of the Mn ion is probably occupied by the sulphonate group of an adjacent complex molecule forming a one-dimensional polymer, as shown in Scheme 2. In this scheme, a planar imino/phenolato N_2O_2 donor set around the Mn ion is proposed for the two complexes, in accordance with the ^1H NMR results (Section 3.3.3). In spite of our numerous efforts, we did not succeed to grow single crystals of good enough quality for an XRD study.

3.2. Solid state studies

3.2.1. FT-IR spectroscopy

Comparison of the IR spectra of 1–2 evidences their similar structures. The IR spectra of the two complexes (Fig. 2) exhibit strong imine and phenolato absorptions at 1613/1611 and 1543 cm^{-1}, two strong bands at 1112 and 1029 cm^{-1} attributable to the asymmetric and symmetric stretching modes of the $-SO_3^-$ group, and a broad O—H stretching band at ≈3480 cm^{-1}. Although $Mn(OAc)_2$ was used in the synthesis of the complexes, the lack of typical acetate stretching bands and the analogous spectrum shown by the complex obtained by reaction of 5-SO_3-salpn with $Mn(ClO_4)_2$ in the presence of base, confirm the absence of acetate in these complexes.

3.2.2. Magnetic properties

The molar magnetic susceptibility (χ_M) of complex 1 in the solid state is independent of the applied magnetic field strength from 0.1 to 2.1 T. The temperature dependence of χ_M was measured in the 2–300 K temperature range on cylindrical 3 mm diameter pellets pressed from powdered 1, under an applied magnetic field of 0.5 T. The room temperature magnetic moment (μ_{eff}) value of 4.87 BM is in excellent accordance with the theoretical value for the spin-only moment of one high-spin Mn^{III} ion (μ_{SO} = 4.90 BM for g = 2). Upon cooling, the μ_{eff} values are practically constant

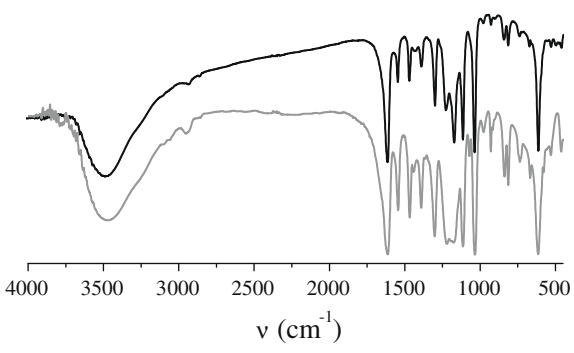


Fig. 2. FT-IR spectra of complexes 1 (—) and 2 (—).

down to 100 K (4.81 BM), then decrease slightly from 100 to ~20 K (4.51), and finally decrease to 3.29 BM at 2 K, indicating operation of either small antiferromagnetic interactions, or single Mn^{III} ion zero field splitting, or both. Plots of the inverse corrected molar susceptibility vs. temperature yielded straight lines, indicating Curie–Weiss behavior, with $C = 2.91\ cm^3\ mol^{-1}\ K$ and $\theta = -6.7$ K. The Curie constant C value is close to the expected value of $3.0\ cm^3\ mol^{-1}\ K$ for an isolated Mn^{III} ion ($S = 2$, $g = 2$), and the negative θ value further confirms the possible operation of weak antiferromagnetic interactions in this complex. Considering that all solid state and solution studies point to the mononuclear nature of 1 and 2, the magnetic behavior of 1 may originate from antiferromagnetic intermolecular spin–spin interactions and/or single ion zero field splitting (zfs) of Mn^{III}. Consequently, we initially fitted the magnetic data by considering the theoretical magnetic susceptibility of an $S = 2$ spin system taking into account an axial zfs term, D. The magnetic susceptibility has been computed by exact calculation of the energy levels associated with the spin Hamiltonian through diagonalization of the full energy-matrix with a general program for axial and rhombic symmetries [35]. Two possibilities were considered, either without or with additional operation of extended intermolecular magnetic interactions (zj') computed within the molecular field approximation [36]. When zj' was set to zero, the fits were very poor; when zj' was allowed to vary, similarly poor fits were obtained with zj' values in the $10^{-8}\ cm^{-1}$ range. In both cases, the resulting D values ($10\ cm^{-1}$ range) were physically meaningless (positive and unreasonably large). Considering that the solid state studies point to the possibility of 1D chains formed through axial coordination of the sulphonate group of adjacent complex molecules, we finally fitted the magnetic data by considering chain interactions. As shown in Fig. 3, the fit obtained when computing the magnetic susceptibility with the assumption of an Heisenberg chains of $S = 2$ spins [37] were fairly good for the parameter values $g = 1.931$, $J\ (cm^{-1}) = -0.16$ and negligible paramagnetic contribution (~10^{-8} range), with an agreement factor $R = \sum[(\chi_M T)_{obs} - (\chi_M T)_{calc}]^2/\sum[(\chi_M T)_{obs}]^2 = 2.9 \times 10^{-4}$.

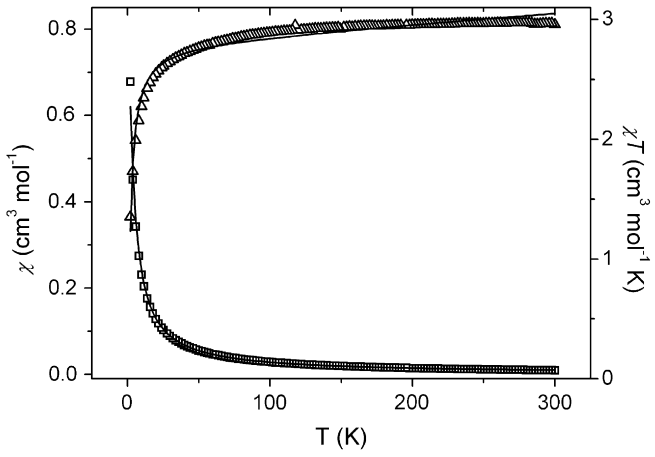


Fig. 3. Temperature dependence of χ_M (□) and $\chi_M T$ (△) for complex 1. The solid lines show the best fit based on the Heisenberg chain model, see text.

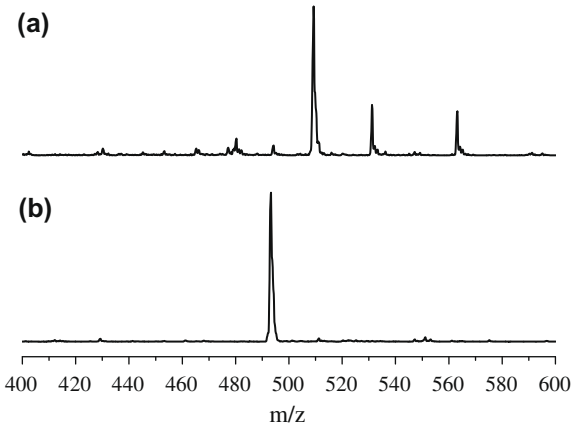


Fig. 4. ESI-MS spectra of 1 (a) and 2 (b).

3.3. Solution studies

3.3.1. Electrospray ionization-mass spectrometry (ESI-MS)

The ESI-mass spectra of 1–2 (Fig. 4) show that both complexes are mononuclear and negatively charged in solution. For complex 1, the parent peak is observed at $m/z = 509.3$ (100%) in the negative mode and originates from the $[Mn(5\text{-}SO_3\text{-}salpnOH)]^-$ monoanion. Two other peaks of lower intensity are also observed at $m/z = 531.5$ (34%) and $m/z = 563.2$ (30%) and can be assigned to $[Mn(5\text{-}SO_3\text{-}salpnO)Na]^-$ and $[Mn(5\text{-}SO_3\text{-}salpnO)Na(MeOH)]^-$. For complex 2, the mass spectrum is dominated by the peak at $m/z = 493.3$ (100%) corresponding to $[Mn(5\text{-}SO_3\text{-}salpn)]^-$. The isotopic patterns of these peaks match very well their simulated spectra.

3.3.2. UV–vis Spectra

The electronic spectra of complexes 1–2 in water exhibit similar features (Fig. 5). The two complexes show an intense absorption at 277 nm which can be attributed to intraligand π–π^* transitions [38,39]. The band at 360/365 nm corresponds to L → M charge-transfer transitions from $p\pi$ orbitals of the phenoxo oxygen to

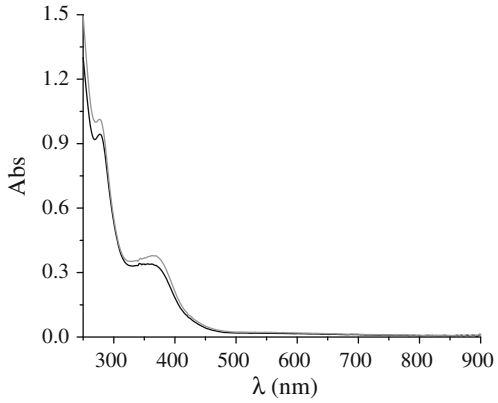


Fig. 5. UV–vis spectra of 1 (▬) and 2 (▬) in water.

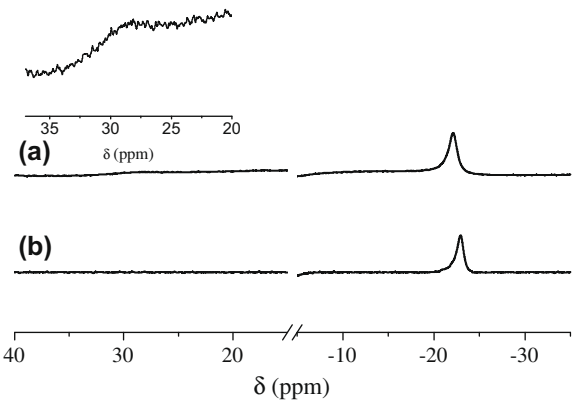


Fig. 6. ^1H NMR spectra of 1 (a) and 2 (b) in D_4-methanol. [complex] ≈ 10 mM.

the partially filled $d\pi$ orbitals of the Mn^{III} ion, as also observed for other Mn^{III} complexes with phenoxo ligands [33,40,41]. Absorption at 545 nm (ε = 397 (1) and 444 (2) M^{-1} cm^{-1}), can be assigned to a *d–d* transition in agreement with the values reported for related Mn^{III} complexes with the metal in a pseudotetragonal environment [42,43].

3.3.3. ^1H NMR spectroscopy

The paramagnetic ^1H NMR spectra of 1 and 2 in D_4-methanol (Fig. 6) revealed a simple pattern for the two complexes outside the diamagnetic region (ca. 0–10 ppm). In both cases, one resonance is observed up-field at δ: −22 (1) and −23 ppm (2), which can be assigned to the H4 aromatic ring proton on the basis of comparison with reported spectra for other phenolato based Mn^{III} complexes [44,45]. Because of their closeness to the Mn centre, protons adjacent to the donor groups of the Schiff base ligand (aromatic H3 and H6, N=CH— and —CH_2—N=C) are not observed, and this is consistent with previous finding for related Mn^{III} complexes [46]. A very broad and weak resonance at +28 ppm (inset of Fig. 6a) is observed in the spectrum of 1. This signal may arise from the carbinolic proton of $[Mn(5\text{-}SO_3\text{-salpnOH})]^-$, in accordance with the resonance at +28.6 ppm reported for $[Mn(salpnOH)(MeOH)_2]^+$ [44], absent in the alkoxo-bridged dimer $[Mn(salpnO)]_2$ [45,47].

NMR has proven to be a useful probe of the ligand conformation for Mn-Schiff-base complexes in solution [44]. ^1H NMR spectra of tetragonal mononuclear Mn complexes with tetradentate Schiff base ligands symmetrically arranged in the equatorial plane show two up-field resonances around −20 to −25 ppm assigned to H4/H4′ and H5/H5′ protons of the phenolato rings [44,48–50]. A more complex pattern is observed in the ^1H NMR spectra of Mn complexes in which the ligand is not symmetrically arranged around the Mn ion because the different extent of charge transfer for protons of the phenolato *trans* to different groups results in the magnetic nonequivalence of protons of the two phenolato rings [44,51]. Therefore, observation of only one resonance for the aromatic ring protons in the ^1H NMR spectra of 1–2 indicates that these complexes possess trans-diaxial symmetry with the N_2O_2 donor set of the ligand located in the equatorial plane.

3.3.4. Electrochemical studies

The electrochemical properties of complexes 1–2 were investigated by cyclic voltammetry in degassed aqueous solutions containing 0.1 M KNO_3. The two complexes exhibit one quasi-reversible reduction wave (shown in Fig. 7) at $E_{1/2}$ −47.11 (1) and −77.80 mV (2), with peak to peak separation (100 mV/s scan rate) slightly above the value expected for a one-electron electrochemically reversible process, and I_{pa}/I_{pc} peak-intensity ratio of 0.96 (1) and 1.12 (2). Plots of I_p vs. $v^{1/2}$ are linear in the 25–250 mV/s range of scan rates (v), indicating a diffusion-controlled process. Voltammetry at a rotating electrode confirmed that the quasi-reversible waves correspond to a reduction process attributable to the Mn^{III}/Mn^{II} couple. No metal-centred oxidation was observed in the anodic scan up to potentials of 1.0 V, in accordance with similar observations for other tetragonal Mn-Schiff-base complexes [39,52,53]. At variance, it has been found that complexes in which salpn adopts the *cis-β* configuration and which contain one additional oxyanion bound to Mn, the potentials of the Mn^{III}/Mn^{IV} and Mn^{III}/Mn^{II} couples are shifted to much lower values associated with the change in donor set and geometry about Mn [45]. Therefore, the electrochemical results confirm that both 5-SO_3-salpn and 5-SO_3-salpnOH bind Mn through the N_2O_2 donor set disposed in one plane.

It has been observed that the catalytic rate constants for dismutation of the superoxide anion are related to the metal-centred reduction potential of Mn^{III} compounds [19]. The closer the approach to the midpoint potential between the reduction and oxidation of $O_2^{\cdot-}$ ($E_{1/2}$ 0.12 V vs. Ag/AgCl) the more potent the mimic. The Mn^{III}/Mn^{II} potential of MnSOD of 8 mV (vs. Ag/AgCl) has been proposed to be one of the causes of its high efficiency [18,19,54–56]. Since the Mn^{III}/Mn^{II} redox couple of 1 and 2 is within the potential range −0.4 V ($O_2/O_2^{\cdot-}$) to 0.65 V ($O_2^{\cdot-}/H_2O_2$), these complexes are expected to be efficient SOD mimics.

3.3.5. SOD activity

In order to evaluate the activity of complexes 1–2 toward superoxide in aqueous buffer, the NBT assay was used. This assay is based on kinetic competition for the superoxide reaction between NBT and the complex with SOD activity. In this way the SOD activity is inversely related to the amount of formazan, the purple product formed by reaction of NBT with superoxide, observed at 560 nm. When added to the reaction mixture, both 1 and 2 were found to inhibit the reduction of NBT, as shown in Fig. 8. Inhibition percentages were measured for several complex concentrations and the IC_{50} values, graphically evaluated, were 1.14 μM (1) and 0.77 μM (2). Comparing the activity of complexes 1 and 2, the presence of the OH group on C2 of the propane backbone of 1 does not seem to contribute to the proton transfer that must assist dismutation of $O_2^{\cdot-}$; on the contrary, it diminishes the SOD activity of the catalyst. In order to compare the SOD activity of complexes 1–2 with that of the water soluble Mn-salen

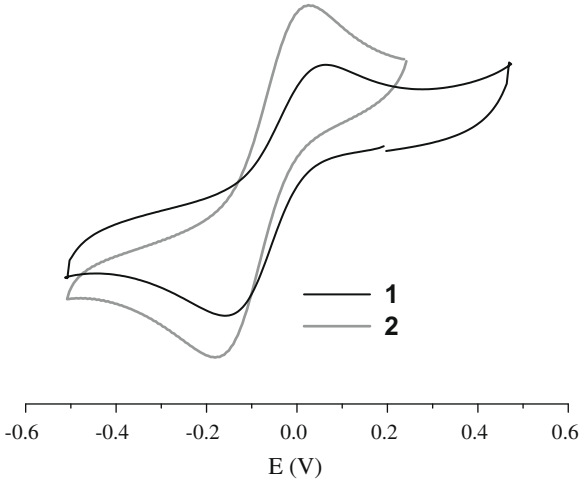


Fig. 7. Cyclic voltammograms of 1–2 in water. Conditions: Pt/Pt/Ag–AgCl; conc. = 1 mM; scan rate = 100 mV s^{-1}; supporting electrolyte = KNO_3.

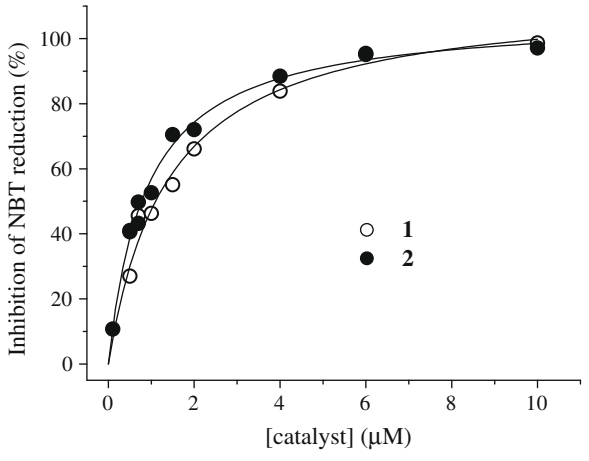


Fig. 8. SOD-activity of complexes 1 and 2 in the riboflavin–methionine–NBT assay.

analogue, we synthesized [Mn(5-SO_3-salen)]$^-$. This complex shows a Mn^{III}/Mn^{II} couple at 205 mV and the IC_{50} value, measured under the same experimental conditions as complexes 1–2, was 2.34 μM. The fact that the catalyst with higher reduction potential reacts slower (2 > 1 > [Mn(5-SO_3-salen)]$^-$), indicates that oxidation of the catalyst with simultaneous $O_2^{\cdot-}$ reduction should be the slow step in the catalytic cycle. Similar correlations were observed for Fe^{II} complexes [57] and Mn^{II} or Mn^{III} complexes of polypodal amines [58], while the opposite occurs with Mn^{III}-porphyrins [18,19,59]. Thus, the more flexible 5-SO_3-salpn and 5-SO_3-salpnOH are better suited than 5-SO_3-salen as Mn-based SOD mimics.

On the basis of competition with NBT, at 50% inhibition the rates of the reactions of NBT and the mimic with $O_2^{\cdot-}$ are equal, k_{cat} [catalyst] = k_{NBT} [NBT], where k_{NBT} (pH = 7.8) = 5.94×10^4 M^{-1} s^{-1} [58,60]. Hence, the catalytic rate constant, $k_{cat} = k_{NBT}$ [NBT]/IC_{50}, were calculated to be k_{cat} 2.4×10^6 (1) and 3.6×10^6 (2) M^{-1} s^{-1}. These values are independent of the detector concentration and appropriate for comparison with literature. The SOD-like activity of complexes 1–2 is higher than those of other MnSOD mimics with open chain ligands [61–65], which clearly indicates that these complexes can be used as catalytic $O_2^{\cdot-}$ scavengers.

3.3.6. Catalase activity

Since H_2O_2 is produced during $O_2^{\cdot-}$ disproportionation by SOD mimics, we decided to investigate the ability of complexes 1–2 to catalyze the removal of H_2O_2 in water solution. The two complexes showed similar behavior, disproportionating up to 100 equivalents of H_2O_2. When the reaction was performed in aqueous borate buffer (pH = 8.0), the two complexes were able to disproportionate up to 250 equivalents of H_2O_2. At this pH, the measured initial turnover rates were 178 (1) and 25.2 (2) mM H_2O_2 min^{-1} mM^{-1} $catalyst^{-1}$. The higher rate observed for 1 is probably related to the presence of the OH group on C2 of the propane backbone that facilitates the formation of the dimer required for CAT activity. Although lower than observed for catalysts that exist as dinuclear or dimeric entities in solution [66–69], the catalytic turnover activity (maximal concentration of oxygen) of these water soluble complexes is ~10–30 times larger than for complexes of the Mn-salen family [25,70], an advantageous property for acting as ROS scavengers.

4. Conclusions

5-SO_3-salpnOH and 5-SO_3-salpn differ in the presence of the OH group at C2 of the propane backbone. However, the two ligands coordinate Mn through the imino/phenolato N_2O_2 donor set, to afford analogous [Mn(SO_3-salpn(OH))]$^-$ with the Mn ion in a tetragonal environment and the Schiff base ligand symmetrically arranged in the equatorial plane. Although the OH group does not affect the geometry of the complex, it influences the Mn^{III}/Mn^{II} redox couple. As a result, complex 2 shows SOD activity higher than 1. Besides, 1–2 are better SOD mimics than [Mn(5-SO_3-salen)]$^-$ and show improved CAT activity over that of complexes belonging to the Mn-salen family [25,70]. These results render these complexes particularly attractive as catalytic antioxidants for pharmaceutical purposes.

Acknowledgements

We thank the National University of Rosario, CONICET and the National Agency for Sciences Promotion for financial support, and CONICET and CNRS for a bilateral agreement (Res. 709/2005). D.M. thanks the France Embassy and MECT of Argentina for a fellowship.

References

[1] A.-F. Miller, Curr. Opin. Chem. Biol. 8 (2004) 162–168.
[2] P.A. Kocatürk, M.C. Akbostanci, F. Tan, G.Ö. Kavas, Pathophysiology 7 (2000) 63–67.
[3] Y. Ihara, M. Chuda, S. Kuroda, T. Hayabara, J. Neurol. Sci. 170 (1999) 90–95.
[4] M.E. De Leo, S. Borrello, M. Passantino, B. Palazzotti, A. Mordente, A. Daniele, V. Filippini, T. Galeotti, C. Masullo, Neurosci. Lett. 250 (1998) 173–176.
[5] S.L. Marklund, R. Adolfsson, C.G. Gottfries, B. Winblad, J. Neurol. Sci. 67 (1985) 319–325.
[6] Y. Toh, S. Kuninaka, M. Mori, T. Oshiro, Y. Ikeda, H. Nakashima, H. Baba, S. Kohnoe, T. Okamura, K. Sugimachi, Oncology 59 (2000) 223–228.
[7] R.H. Burdon, Free Radic. Biol. Med. 18 (1995) 775–794.
[8] F. Yamakura, K. Kobayashi, H. Ue, M. Konno, Eur. J. Biochem. 227 (1995) 700–706.
[9] H.M. Steinman, L. Weinstein, M. Brenowitz, J. Biol. Chem. 269 (1994) 28629–28634.
[10] M.L. Ludwig, A.L. Metzger, K.A. Pattridge, W.C. Stallings, J. Mol. Biol. 219 (1991) 335–358.
[11] R.A. Edwards, H.M. Baker, M.M. Whittaker, J.W. Whittaker, G.B. Jameson, E.N. Baker, J. Biol. Inorg. Chem. 3 (1998) 161–171.
[12] G.E.O. Borgstahl, H.E. Parge, M.J. Hickey, W.F. Beyer, R.A. Hallewell, J.A. Tainer, Cell 71 (1992) 107–118.
[13] L. Noodleman, T. Lovell, W.G. Han, J. Li, F. Himo, Chem. Rev. 104 (2004) 459–508.
[14] L.E. Grove, J. Xie, E. Yikilmaz, A. Karapetyan, A.-F. Miller, T.C. Brunold, Inorg. Chem. 47 (2008) 3993–4004.
[15] C. Muscoli, S. Cuzzocrea, D.P. Riley, J.L. Zweier, C. Thiemermann, Z.-Q. Wang, D. Salvemini, Br. J. Pharmacol. 140 (2003) 445–460.
[16] A. Gärtner, U. Weser, in: E. Vögtle, E. Weber (Eds.), Topics in Current Chemistry: Biomimetic and Bioorganic Chemistry II, vol. 132, Springer-Verlag, Berlin, 1986, pp. 1–61.
[17] P.K. Gonzalez, J. Zhuang, S.R. Doctrow, B. Malfroy, P.F. Benson, M.J. Menconi, M.P. Fink, J. Pharmacol. Exp. Ther. 275 (1995) 798–806.
[18] I. Batinic-Haberle, I. Spasojevi, R.D. Stevens, P. Hambright, P. Neta, A. Matsumoto, I. Fridovich, Dalton Trans. (2004) 1696–1702.
[19] I. Batinic-Haberle, I. Spasojevic, P. Hambright, L. Benov, A.L. Crumbliss, I. Fridovich, Inorg. Chem. 38 (1999) 4011–4022.
[20] I. Batinic-Haberle, I. Spasojevic, R.D. Stevens, B. Bondurant, A. Okado-Matsumoto, I. Fridovich, Z. Vujaskovic, M.W. Dewhirsta, Dalton Trans. (2005) 617–624.
[21] R.A. Rosenthal, K.D. Huffman, L.W. Fisette, C.A. Damphousse, W.B. Callaway, B. Malfroy, S.R. Doctrow, J. Biol. Inorg. Chem. 14 (2009) 979–991.
[22] I. Pálinkó, in: J.G. Huges, A.J. Robinson (Eds.), Inorganic Biochemistry Research Progress, Nova Science Publishers Inc., New York, 2008, pp. 281–303.
[23] D.P. Riley, S.L. Henke, P.J. Lennon, R.H. Weiss, W.L. Neumann, W.J. Rivers, K.W. Aston, K.R. Sample, H. Rahman, C.-S. Ling, J.-J. Shieh, D.H. Busch, Inorg. Chem. 35 (1996) 5213–5231.
[24] A. Maroz, G.F. Kelso, R.A.J. Smith, D.C. Ware, R.F. Anderson, J. Phys. Chem. A 112 (2008) 4929–4935.
[25] S.R. Doctrow, K. Huffman, C.B. Marcus, C. Tocco, E. Malfroy, C.A. Adinolfi, H. Kruk, K. Baker, N. Lazarowych, J. Mascarenhas, B. Malfroy, J. Med. Chem. 45 (2002) 4549–4558.
[26] K.J. Berry, F. Moya, K.S. Murray, A.M.B. Bergen, B.O. West, J. Chem. Soc., Dalton Trans. (1982) 109–116.
[27] M. Botsivali, D.F. Evans, P.H. Missen, M.W. Upton, J. Chem. Soc., Dalton Trans. (1985) 1147–1149.
[28] P. Pascal, Ann. Chim. Phys. 19 (1910) 5–70.
[29] F. James, M. Roos, Comput. Phys. Commun. 10 (1975) 343–367.
[30] C. Beauchamps, I. Fridovich, Anal. Biochem. 44 (1971) 276–287.
[31] C. Palopoli, B. Chansou, J.-P. Tuchagues, S. Signorella, Inorg. Chem. 39 (2000) 1458–1462.
[32] M. Maneiro, M.R. Bermejo, A. Sousa, M. Fondo, A.M. González, A. Sousa-Pedrades, C.A. McAuliffe, Polyhedron 19 (2000) 47–54.
[33] J.W. Gohdes, W.H. Armstrong, Inorg. Chem. 31 (1992) 368–373.
[34] H. Shyu, H. Wei, Y. Wang, Inorg. Chim. Acta 290 (1999) 8–13.
[35] A.K. Boudalis, J.-M. Clemente, F. Dahan, J.-P. Tuchagues, Inorg. Chem. 43 (2004) 1574–1586.
[36] A.P. Ginsberg, M.E. Lines, Inorg. Chem. 11 (1972) 2289–2290.
[37] K. Konig, V.P. Desai, B. Kanellakopulos, R. Klenze, Chem. Phys. 54 (1980) 109–113.
[38] A. Neves, S.M.D. Erthal, I. Vencato, A.S. Ceccato, Y.P. Mascarenhas, O.R. Nascimento, M. Horner, A.A. Batista, Inorg. Chem. 31 (1992) 4749–4755.
[39] X. Li, V.L. Pecoraro, Inorg. Chem. 28 (1989) 3403–3410.
[40] K. Bertoncello, G.D. Fallon, K.S. Murray, E.R.T. Tiekink, Inorg. Chem. 30 (1991) 3562–3568.
[41] D. Pursche, M.U. Triller, C. Slinn, N. Reddig, A. Rompel, B. Krebs, Inorg. Chim. Acta 357 (2004) 1695–1702.
[42] A.B.P. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, 1984. pp. 440–444.
[43] F.M. Ashmawy, C.A. McAuliffe, R.V. Parish, J. Tames, J. Chem. Soc., Dalton Trans. (1985) 1391–1397.
[44] J.A. Bonadies, M.J. Maroney, V.L. Pecoraro, Inorg. Chem. 28 (1989) 2044–2051.
[45] E.J. Larson, V.L. Pecoraro, J. Am. Chem. Soc. 113 (1991) 3810–3818.
[46] M. Hoogenraad, K. Ramkisoensing, W.L. Driessen, H. Kooijman, A.L. Spek, E. Bouwman, J.G. Haasnoot, J. Reedijk, Inorg. Chim. Acta 320 (2001) 117–126.

[47] J.A. Bonadies, M.L. Kirk, M.S. Lah, D.P. Kessissoglou, W.E. Hatfield, V.L. Pecoraro, Inorg. Chem. 28 (1989) 2037–2044.
[48] M.T. Caudle, P. Riggs-Gelasco, A.K. Gelasco, J.E. Penner-Hahn, V.L. Pecoraro, Inorg. Chem. 35 (1996) 3577–3584.
[49] M.R. Bermejo, A.M. González, M. Fondo, A. García-Deibe, M. Maneiro, J. Sanmartín, O. Hoyos, M. Watkinson, New J. Chem. 24 (2000) 235–241.
[50] M.R. Bermejo, A.M. González-Noya, V. Abad, M.I. Fernández, M. Maneiro, R. Pedrido, M. Vásquez, Eur. J. Inorg. Chem. (2004) 3696–3705.
[51] D.P. Kessissoglou, W.M. Butler, V.L. Pecoraro, Inorg. Chem. 26 (1987) 495–503.
[52] E. Larson, M.S. Lah, X. Li, J.A. Bonadies, V.L. Pecoraro, Inorg. Chem. 31 (1992) 373–378.
[53] R.K. Boggess, J.W. Hughes, W.M. Coleman, L.T. Taylor, Inorg. Chim. Acta 38 (1980) 183–189.
[54] W.C. Barrette, D.T. Sawyer, J.A. Fee, K. Asada, Biochemistry 22 (2002) 624–627.
[55] W.H. Koppenol, F. Levine, T.L. Hatmaker, J. Epp, J.D. Rush, Arch. Biochem. Biophys. 251 (1986) 594–599.
[56] J. Stein, J.P.J. Fackler, G.J. McClune, J.A. Fee, L.T. Chan, Inorg. Chem. 18 (1979) 3511–3519.
[57] P.J.F. Gauuan, M.P. Trova, L. Gregor-Boros, S.B. Bocckino, J.D. Crapo, B.J. Day, Bioorg. Med. Chem. 10 (2002) 3013–3021.
[58] S. Durot, C. Policar, F. Cisnetti, F. Lambert, J.-P. Renault, G. Pelosi, G. Blain, H. Korri-Youssoufi, J.-P. Mahy, Eur. J. Inorg. Chem. (2005) 3513–3523.
[59] R. Kachadourian, I. Batinic-Haberle, I. Fridovich, Inorg. Chem. 38 (1999) 391–396.
[60] Z.R. Liao, X.F. Zheng, B.S. Luo, L.R. Shen, D.F. Li, H.L. Liu, W. Zhao, Polyhedron 20 (2001) 2813–2821.
[61] D.P. Riley, Chem. Rev. 99 (1999) 2573–2588.
[62] Q.X. Li, Q.H. Luo, Y.Z. Li, Z.Q. Pan, M.C. Shen, Eur. J. Inorg. Chem. (2004) 4447–4456.
[63] E.A. Lewis, H.H. Khodr, R.C. Hilder, J.R. Smith, P.H. Walton, Dalton Trans. (2004) 187–188.
[64] J. Lin, C. Tu, H. Lin, P. Jiang, J. Ding, Z. Guo, Inorg. Chem. Comm. 6 (2003) 262–265.
[65] U.P. Singh, A.K. Sharma, P. Tyagi, S. Upreti, R.K. Singh, Polyhedron 25 (2006) 3628–3638.
[66] A. Gelasco, S. Bensiek, V.L. Pecoraro, Inorg. Chem. 37 (1998) 3301–3309.
[67] H. Biava, C. Palopoli, S. Shova, M. De Gaudio, V. Daier, M. González-Sierra, J.-P. Tuchagues, S. Signorella, J. Inorg. Biochem. 100 (2006) 1660–1671.
[68] D. Moreno, C. Palopoli, V. Daier, S. Shova, L. Vendier, M. González-Sierra, J.-P. Tuchagues, S. Signorella, Dalton Trans. 10 (2006) 5156–5166.
[69] S. Signorella, A. Rompel, K. Buldt-Karentzopoulos, B. Krebs, V.L. Pecoraro, J.-P. Tuchagues, Inorg. Chem. 46 (2007) 10864–10868.
[70] W. Park, D. Lim, Bioorg. Med. Chem. Lett. 19 (2009) 614–617.