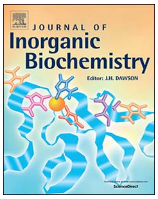
Journal of Inorganic Biochemistry 182 (2018) 29–36

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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



Tuning the Mn_2^{II}/Mn_2^{III} redox cycle of a phenoxo-bridged diMn catalase mimic with terminal carboxylate donors

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ARTICLE INFO

Keywords:
Catalase mimic
Manganese
Kinetics
Polycarboxylate ligand

ABSTRACT

A new phenoxo-bridged $diMn^{III}$ complex, $Na[Mn_2L(OH)_2(H_2O)_2]·5H_2O$ (**1**), obtained with the ligand L^{5-} = 5-methyl-2-hydroxo-1,3-xylene-α,α-diamine-*N*,*N*,*N′*,*N′*-tetraacetato, has been prepared and characterized. Mass spectrometry, conductivity, UV–visible, EPR and ^1H NMR spectroscopic studies showed that the complex exists in solution as a monoanionic $diMn^{III}$ complex. Complex **1** catalyzes H_2O_2 disproportionation with second-order rate constant k_{cat} = 305(9) M^{-1} min^{-1} and without a time-lag phase. Based on spectroscopic results, the catalase activity of complex **1** in methanol involves a Mn_2^{III}/Mn_2^{II} redox cycle, which distinguishes this catalyst from other phenoxo-bridged diMn complexes that cycle between $Mn^{II}Mn^{III}/Mn^{III}Mn^{IV}$ species. Addition of base stabilizes the catalyst, restrains demetallation during catalysis and causes moderate enhancement of catalase activity. The terminal carboxylate donors of **1** not only contribute as internal bases to assist deprotonation of H_2O_2 but also favor the formation of active homovalent diMn species, just as observed for the enzyme.

1. Introduction

Manganese catalases (MnCAT) catalyze the disproportionation of harmful H_2O_2 into H_2O and O_2 by using a $Mn_2(\mu\text{-}O_2CR)(\mu\text{-}O/OH/H_2O)_2$ structural unit as the active site [1,2], with terminal carboxylates bound to the Mn ions to assist the proton transfer during catalysis [3]. The highly efficient dismutation of H_2O_2 by MnCAT requires a two-electron redox cycle that involves Mn_2^{II} and Mn_2^{III} oxidation levels [4,5]. When using artificial compounds to model the enzymatic activity, the fine-tuning of Mn redox states is a critical feature [6]. The number and type of ligands, the total charge, the nuclearity of the site, are among the factors that introduce a way of tuning the oxidation states of the metal center to face the redox reaction. Owing to their potential use as catalytic scavengers of H_2O_2 for preventing oxidative stress damage, many diMn complexes have been tested as MnCAT mimics [7,8]. Dinucleating ligands with a central alkoxo or phenoxo group afford a scaffold to enhance co-operativity between the two Mn ions and have been used to model the active site of MnCAT. While alkoxo-bridged diMn complexes decompose H_2O_2 through catalytic cycles involving homovalent Mn_2^{II}/Mn_2^{III} or Mn_2^{III}/Mn_2^{IV} active species [6–8], phenoxo-bridged diMn complexes of heptadentate ligands tested to date, employ mixed valence diMn species during catalysis [7,9–11]. $Mn^{II}Mn^{III}/Mn^{III}Mn^{IV}$ oxidations states were detected during disproportionation of H_2O_2 by phenoxo-bridged diMn complexes of ligands bearing terminal pyridyl [10], pyridyl/benzyl [9] and pyridyl/carboxylate [10] groups, instead of Mn_2^{II}/Mn_2^{III} states employed by the biosite. With the aim of determining the effect of replacing all terminal pyridyl by carboxylato donors on the active oxidation states of phenoxo-bridged diMn CAT mimics, we report here the synthesis, characterization and CAT activity, of a diMn complex with an *O*-rich coordination sphere $Na[Mn_2L(OH)_2(H_2O)_2]·5H_2O$ (**1**·$5H_2O$), obtained with the N_2O_5-ligand L^{5-} = 5-methyl-2-hydroxo-1,3-xylene-α,α-diamine-*N*,*N*,*N′*,*N′*-tetraacetato, and compare its catalytic activity with other phenoxo-bridged diMn models. Besides, we show that addition of an exogenous base stabilizes the catalyst and has moderate effect on the CAT activity thus confirming the role played by terminal carboxylato donors to assist proton transfer during H_2O_2 dismutation.

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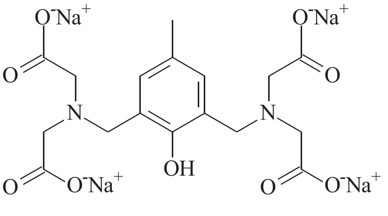
https://doi.org/10.1016/j.jinorgbio.2018.01.013
Received 24 October 2017; Received in revised form 14 January 2018; Accepted 21 January 2018



Na_4HL

2. Experimental section

2.1. Materials

All reagents or AR chemicals were used as purchased. Solvents were purified by standard methods. The concentration of H_2O_2 stock solution was determined by iodometric titration.

2.2. Synthesis of ligand and complex

2.2.1. Tetra sodium salt of 5-methyl-2-hydroxy-1,3-xylene-α,α-diamine-N,N,N′,N′-tetraacetic acid (Na_4HL)

The Na_4HL ligand was synthesized by a modification of the procedure of Schwarzenbach [12,13]. To a mixture of a 6.5 mL aqueous solution of 836.4 mg (6.28 mmol) of iminodiacetic acid and 2 mL aqueous solution of 346.8 mg (3.2 mmol) of *p*-cresol cooled in an ice-water bath were added 2 mL of 6.25 M NaOH. Upon dissolution, 0.75 mL of 37% formaldehyde was added dropwise. The solution was stirred for 30 min at 0 °C, heated at reflux for 5 h, and then concentrated to dryness. The solid was recrystallized from a 2:1 methanol:ethanol mixture. Colorless crystals of Na_4HL were filtered off, washed with ether and dried under vacuum. Yield: 1.3078 g (86%). ^1H NMR in D_2O, δ (ppm): 2.14 (s, 3H), 3.10 (s, 8H, N-$\underline{CH_2}$-CO_2); 3.65 (s, 4H, Ar-$\underline{CH_2}$-N), 6.91 (s, 2H, $\underline{H}_{Ar}$). ^{13}C NMR in D_2O, δ (ppm): 19.30 (Me); 55.03 (Ar-$\underline{C}$-N); 57.95 (N-$\underline{C}$-CO_2); 123.15 (C1/C3); 128.60 (C5); 131.38 (C4/C6); 158.10 ($\underline{C}_{Ar}$-OH); 179.00 ($\underline{CO_2}^-$). IR (KBr, ν cm^{-1}): 3422, 2923, 2826, 1594, 1481, 1411, 1328, 861, 725. Mp: 270–271 °C. UV–vis, $\lambda_{máx}$ nm (ε M^{-1} cm^{-1}) in H_2O: 289 (3900), 315 (sh); in methanol: 290 (2140).

2.2.2. $Na[Mn_2L(OH)_2(H_2O)_2]\cdot 5H_2O$ ($\mathbf{1}\cdot 5H_2O$)

To a solution of 52 mg (0.1069 mmol) of Na_4HL in 2.5 mL MeOH and 0.15 mL distilled H_2O, were added 75 μL (0.5372 mmol) of Et_3N and then a solution of 80.5 mg (0,2224 mmol) Mn $(ClO_4)_2\cdot 6H_2O$ in 1 mL H_2O. The reaction mixture was left with stirring during two days at room temperature. The solid was filtered off, and 20 mL of MeCN were added to the fíltrate, after which the complex began to precipitate. The suspension was left one day without stirring and the solid decanted. The supernatant solution was separated and the solid was washed with ethyl ether and dried under vacuum. Yield: 34 mg (58.6%). Anal. calcd for $C_{17}H_{23}Mn_2N_2NaO_{13}\cdot 5H_2O$: C: 29.8, H 4.8, Mn 16.0, N 4.1%; Found: C 29.5; H 4.6; Mn 16.2; N 4.1%. UV–vis, $\lambda_{máx}$ nm (ε M^{-1} cm^{-1}) in MeOH: 239 (9962), 283 (6415), 380 (sh), 602 (190); in MeOH/Et_3N: 236 (17400), 276 (11630), 390 (5110). Significant IR bands (KBr, ν cm^{-1}): ν_{OH} 3425 (broad), $\nu_{C\text{-}H}$ 2922/2870,ν_{CO2-} 1602/1405, $\nu_{C\text{-}O/N}$ 1323, 1262. The content of five molecules of non-coordinated water per complex molecule was confirmed by thermogravimetric analysis of the complex which showed 13.5% mass loss below 150 °C. Molar conductivity = 96 Ω^{-1} cm^2 mol^{-1}.

2.3. Physical and analytical 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. Mass spectra were recorded on an ion trap mass spectrometer (ThermoScientific LCQ DECA XP Max) equipped with an electrospray ionization source (ESI-MS). The solutions for mass spectrometry were prepared from solutions of the complexes or reaction mixtures and diluted with methanol to a 100 μM concentration and directly infused at a flow rate of 10 μL min^{-1}. Melting point was taken on a Fisher-Johns (Ionomex) apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300 NMR spectrometer at ambient probe temperature (ca. 26 °C). Paramagnetic NMR spectra were acquired using super WEFT sequence; with acquisition time of 67 ms. Magnetic susceptibility data were collected with a Quantum Design MPMS SQUID susceptometer. EPR spectra were obtained on a Bruker ESP 300 E spectrometer with a microwave frequency generated with a Bruker ER 04 (9–10 GHz). TGA measurements were conducted on a Perkin-Elmer Diamond TG/DTA Instrument. The compound was heated at the rate of 10 °C/min between RT and 1200 °C. Conductivity measurements were performed using a Horiba F-54 BW conductivity meter, on 1.0 mM solutions of the complexes in methanol. The Mn content was measured by atomic absorption on a METROLAB 250 AA spectrophotometer. The Na content was determined by atomic absorption on an UNICAM spectrophotometer, model 969, at 5890 Å. 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 aqueous buffered solutions (50 mM Et_3N–Et_3NH^+, pH = 10) using 0.1 M $NaNO_3$ 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. Potentials are referred to the Ag/AgCl/sat KCl electrode. Under these conditions, the reduction peak of the Zobell's solution was observed at 229 mV (vs Ag/AgCl/sat KCl).

2.4. Evaluation of CAT activity

Reaction rates were determined by volumetric measurement of the O_2 evolved after addition of excess of H_2O_2 to a solution of the complex. A round-bottom flask with a rubber septum, containing the degassed solution of the complex, was thermostated at 25 °C and connected to a gas-measuring burette (precision of 0.1 mL). Previously thermostated H_2O_2 was injected through the septum to the stirred complex solution, and the evolved O_2 was measured with the burette. The initial reaction rates were obtained by fitting the $[O_2]$ versus time data to a polynomial expression [14] and calculating the slope of the tangent at time zero. Each rate constant reported here represents the mean value of multiple determinations that fall within ± 5%. All experiments were carried out at 25 °C. Blanc experiments performed with 125 mM H_2O_2 in 50 mM Et_3N without the catalyst showed that after 2 h only 2% of the initial H_2O_2 had disproportionated, while when the catalyst (0.5 mM) was present, all H_2O_2 decomposed in 20 min. When 1.5 mg MnO_2 were used instead of catalyst (all other components the same: $[H_2O_2]$ = 125 mM, $[Et_3N]$ = 50 mM, 5 mL MeOH), the initial rate of H_2O_2 decomposition was 0.3 mM/min, and only 19% of H_2O_2 was decomposed in 2 h.

2.5. Calculations

Geometry optimizations were performed using the program SIESTA [15] within the DFT framework using the Perdew–Burke–Ernzerhof exchange – correlation functional [16]. For all atoms, basis sets of double- ζ plus polarization quality were employed, with a pseudoatomic orbital energy shift of 25 meV and a grid cutoff of 150 Ry [15]. This combination of functional, basis sets, and grid parameters was validated previously [17]. Starting geometry was built in-silico from the structure of $Mn_2[(FHXTA)(H_2O)_4]^-$ (where H_5F-HXTA = 5-fluoro-2-hydroxy-1,3-xylene-α,α′-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) [18]. Atomic coordinates are given in Table S1.