Dinuclear Zn Complex: Phenoxy Radical Formation Driven by Superoxide Coordination

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Dedicated to Professor Dr. Peter Klüfers on the Occasion of his 70th Birthday

Introduction

Molecular dioxygen $O_2$ is a fundamental component for aerobic life. However, during the biological processes, $O_2$ is converted into reactive oxygen species (ROS) including the superoxide radical anion ($O_2^{•−}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical (HO$^•$). Once generated, the superoxide radical might react with redox active metal centers starting a cascade of reactions that lead to the formation of HO$^•$, a highly reactive species responsible for the unselective oxidation of proteins, DNA and other biomolecules. These phenomena are responsible for the so-called “oxidative stress”, a condition strongly related to several pathologies like Alzheimer and cancer.\[1,2]\ The natural defence against ROS is provided by the combined action of antioxidant enzymes. A cascade detoxification is initiated by superoxide dismutases (SODs) via $O_2^{•−}$ dismutation to $O_2$ and $H_2O_2$ and completed by catalases and glutathione peroxidase, to convert $H_2O_2$ to $O_2$.\[3]\ In particular the Cu,Zn-SOD is an enzyme based on the Cu(II) active center that efficiently dismutate $O_2^{•−}$ cycling between the Cu$^{II}$/Cu$^{I}$ redox states.\[4,5] The Enzyme instead appears to play a role in the overall folding stability and facilitates a broader pH independence. However, the first example of a Zn-based complex, bearing a redox active ligand able to catalytically dismutate superoxide has been recently reported.\[5]\ Another well-established function of the Zn(II) center in the Cu,Zn-SOD, is to act as Lewis acid. In fact, upon binding of the superoxide to form a Zn–$O_2^{•−}$ adduct, both the processes of reduction and oxidation of the superoxide become accelerated.\[6]\ Herein the reactivity of the Zn$_2$L$_2$ complex, a structural analogue of the highly reactive SOD mimic Cu$_2$L$_2$, with $O_2^{•−}$ has been investigated in order to evaluate the interaction and the contribution of the Zn center in the process of superoxide activation.\[7,8]\

Results and Discussion

The ligand HL (2-[[di(2-pyridyl)methyl](methyl)amino)methyl]phenol) has been synthesized according to the literature procedures.\[9]\ This ligand has been recently reported in the literature to form mono and dimeric complexes with first row transition metals (Mn, Cu, Fe) exhibiting excellent properties in the processes of $H_2O_2$ and $O_2^{•−}$ dismutation and proton reduction.\[7,9]\ The tetradentate $N$_4$O$ donor-set provided by the ligand can bind Zn(II) with a bis-pyridyl, tertiary amine and phenolate terminals. The Zn$_2$L$_2$ complex has been obtained by reacting the ligand HL with zinc perchlorate and triethylamine in methanol (Scheme 1).\[7,9]\ Colourless crystals suitable for X-ray analysis have been isolated upon addition of acetonitrile and cooling to 4°C. The
Figure 1. X-ray crystal structure of Zn$_2$L$_2$ (for clarity, counter ions, which are not part of the Zn coordination spheres, have been omitted).

Figure 2. Decay of an initially formed phenoxyl radical intermediate in the reaction between Zn$_2$L$_2$ (25 μM) and an excess of KO$_2$ (4 mM) in DMSO monitored by stopped flow (10 s).
Figure 3. CVs of O$_2$/O$_2^*$ (top) and Zn$_2$L$_2$ (bottom, 0.5 mM) + 1 eq KO$_2$ in acetonitrile (TEABF$_4$, 0.1 M). The redox potentials are referred to Fc/Fc$^+$. 

(ca. 10 min), after the decay of the phenoxyl band, a similar trend can also be observed for Zn$_2$L$_2$ (Figure S6). The UV-vis band, associated to the phenoxyl radical (425 nm, $\epsilon = 7.5 \cdot 10^4$), has been also obtained upon addition of 1 eq of ceric ammonium nitrate (CAN) to an acetonitrile solution of the complex (Figure S7). The radical generated in this way exhibits a longer lifetime ($\tau = 25$ min) in comparison with the one generated in presence of an excess of KO$_2$. The lifetime of the latter is expected due to the additional reactions in which the phenoxyl radical may be involved in the presence of a superoxide excess.$^{[13]}$ However, the high reactivity of the phenoxyl radical does not allow its detection by MS, even when it has been directly generated with CAN.

Once formed, the phenoxyl radical in fact can be reduced again to phenolate by an additional equivalent of superoxide or couple with another phenoxyl or superoxide radical generating the rise of the broad band observed below 400 nm (Figure S6). The UV-vis spectrum of the Zn-center in fact acts as a Lewis acid, tuning the redox properties of the coordinated substrate and in our case allows the electrochemical reduction of superoxide to peroxide and the chemical oxidation of the phenolate to phenoxyl radical. The phenol oxidation in particular appears very interesting considering that this process usually requires very strong chemical oxidants like CAN.$^{[13]}

A further confirmation of the effective coordination of the O$_2^*$ to the Zn-center comes from CW X-band EPR measurements. Adding 5 eq of KO$_2$ (2.5 per Zn center) at room temperature to a Zn$_2$L$_2$ solution (1 mM) in DMSO, and immediately freezing the solution in liquid nitrogen, the formation of an intense signal characteristic for a Zn-superoxo intermediate has been detected.$^{[6]}$ The EPR spectrum measured at 95 K shows the formation of the desired Zn(II)--O$_2^*$ complex overlapped with the spectrum of the free KO$_2$, added in excess (Figure 4a, blue trace). Collecting the EPR spectrum at higher temperature (240 K, Figure 4a green trace) only the anisotropic signal associated to the coordinated superoxide Zn(II)--O$_2^*$ has been detected. This allowed us to simulate the EPR spectrum and to determine the $g$-values for the coordinated superoxide ($g_{||} = 2.06$ and $g_{\perp} = 2.01$ (Figure 4b), which are significantly smaller than those measured for the metal-free O$_2^*$ ($g_{||} = 2.12$ and $g_{\perp} = 2.00$, Figure S13), due to the binding to the Zn(II) ion.$^{[17]}$ By way of comparison, 1 eq of CAN has been added to the complex solution in acetonitrile to promote the phenoxyl radical formation. The obtained EPR spectrum of the frozen solution (Figure S14), shows an isotropic signal ($S = 1/2$) $g_{iso} = 2.00$, associated to the phenoxyl radical.$^{[11]}$ The marked differences between the EPR spectra obtained with KO$_2$ and CAN further confirm the formation of two distinct species and exclude a significant contribution of phenoxyl radical in the experimental spectrum of the Zn(II)--O$_2^*$ complex under these experimental conditions. The deviation observed for the $g_{||}$ value from the free spin value ($g_{||} = 2.0023$) is caused by the spin-orbit interaction as given in Eq. 1 when $\Delta E \gg \lambda$. Where $\lambda$ is the spin–orbit coupling constant (0.014 eV) and $\Delta E$ is the energy splitting between the 2$p\pi_x^*$ and the 2$p\pi_y^*$ levels of the superoxide due to the metal binding.$^{[17,18]}

\[ g_{||} = g_0 + 2\lambda/\Delta E \] (1)

Accordingly, the $g_{||}$ value obtained can be used to determine the $\Delta E$ value for the superoxide in the Zn--O$_2^*$ complex, corresponding in our case to 0.49 eV. This value that increases with the Lewis acidity of the metal center, is in line with the values reported for some mononuclear Zn-superoxo complexes based on pyridinic ligands (0.48–0.87 eV), and is higher in comparison with the value reported for the metal-free O$_2^*$ (0.27 eV).$^{[6,17]}$
In summary, a new dinuclear zinc complex $\text{Zn}_2\text{L}_2$ has been synthesized and fully characterized. The reaction of the complex with superoxide has been addressed by UV-vis, CV, EPR and CSI-MS revealing the formation of a $\text{Zn}_2\text{L}_2\text{O}_2^{* -}$ intermediate. The coordinated superoxide is activated by the Lewis acidity of the Zn center and its reduction comes out to be more favorable in comparison with the free superoxide, as evidenced by CV and EPR analysis. In addition, the inner-sphere ET occurring between the ligand and the coordinated $\text{O}_2^{* -}$ allows the oxidation of the phenolic moiety of the ligand to phenoxyl radical, a process that cannot occur via an outer-sphere ET due to the high redox potential of the phenolate/phenoxyl radical couple. These results pave the way for the reactivity study of the $\text{Zn}_2\text{L}_2\text{O}_2^{* -}$ intermediate obtained, in order to determine its electrophilic properties and evaluate its activity in the oxidation of external substrates like phosphines and other standard organic molecules.

**Conclusions**

Thus, the EPR results follow the CV data confirming a more favorable reduction of the coordinated superoxide thanks to the Lewis acidity of the Zn center (Figure 3).

Finally, the CSI-MS analysis has been performed on a $\text{Zn}_2\text{L}_2$ solution in acetonitrile, upon addition of an excess of solid KO$_2$ (see Experimental Section), in order to determine the nuclearity of the $\text{ZnO}_2^{* -}$ complex. The experiment highlights the formation of a dinuclear Zn-superoxo complex $\text{Zn}_2\text{L}_2\text{O}_2^{* -}$ with $m/z = 768.1368$ (Figure S12). The retention of the dinuclear structure in presence of the coordinated superoxide is remarkable and constitute, to the best of our knowledge, the first example of dinuclear-Zn-superoxide complex reported up to date.

**Experimental Section**

**Materials and Methods:** Commercially available reagents were used directly, unless otherwise noted. The ligand precursors 2-[[di(2-pyridyl)methyl][methyl]amino]methylphenol (HL) was synthesized according to the literature procedures.[9]

UV-Vis spectra were recorded with an Analytik Jena AG Specord 200 UV-VIS-NIR spectrometer. FT-IR spectra were recorded Shimadzu ATR MIRacle 10 as solid samples from a diamond crystal. Cyclic voltammetry measurements were performed using an Autolab instrument with a PGSTAT 101 potentiostat. A three-electrode arrangement was employed consisting of a glassy carbon disk working electrode (A = 0.07 cm$^2$) (Metrohm), a platinum counter electrode (Metrohm) and a silver wire (Metrohm) as reference electrode. Potentials were referred to a Fc/Fc$^+$ redox couple. Prior to each experiment, the electrode was polished with 1 μm alumina, rinsed with deionised water and wiped with a paper tissue. The CSI-MS measurements were performed on a UHR-TOF Bruker Daltonik maXis plus, an ESI-quadrupole time-of-flight (qToF) mass spectrometer capable of a resolution of at least 60,000 (FWHM), which was coupled to a Bruker Daltonik Cryospray unit. Detection was in positive ion mode, the source voltage was 3.5 kV and the flow rate was 240 μl/hour. The temperature of the spray gas (N$_2$) was held at −40 °C and the temperature of the dry gas for solvent removal was kept at −35 °C. The mass spectrometer was calibrated prior to every experiment via direct infusion of Agilent ESI-TOF low concentration tuning mixture, which provided an $m/z$ range of singly charged peaks up to 2700 Da in both ion modes. Applied solvents were not extra dry and could provide a source of protons. Processing of the measured data was done with Bruker DataAnalysis. EPR spectra were recorded on a JEOL continuous wave spectrometer JES-FA200, equipped with an X-band Gunn diode oscillator bridge, a cylindrical mode cavity, and an N$_2$ cryostat. The samples were measured in...
solution, under nitrogen atmosphere, in quartz glass EPR tubes at 95 and 240 K, respectively. The spectra shown were measured using the following parameters: microwave frequency $\nu = 8.959$ GHz, modulation width 0.1 mT, microwave power 1.0 mW, modulation frequency 100 kHz, time constant 0.1 s. Analysis and simulation of the data was carried out using the software “eview” and “esim”, written by E. Bill (MP) for Chemical Energy Conversion, Mülheim an der Ruhr).[24]

**Caution** ! Although the salts and the complexes reported do not appear to be mechanically sensitive, perchlorates should be treated with due caution.

[Zn$_2$(MeOH)$_2$](ClO$_4$)$_2$; 73 mg (0.20 mmol) of Zn(ClO$_4$)$_2$·6H$_2$O and ligand 2-[[Di(2-pyridyl)methyl](methyl)amino)methyl]phenol (60 mg, 0.20 mmol), were dissolved 4 mL of methanol. Subsequently, triethylamine was added (28.0 µL, 0.20 mmol) and after one hour stirring a white solid precipitated from the reaction mixture. Acetonitrile was added until complete dissolution of the solid and the solution stored in the fridge at 4°C. After a week, the colourless crystals obtained were filtered and thoroughly washed with cold methanol and ether and finally dried under vacuum. Yield: 51 mg, 60%.

**UV-Vis** (CH$_2$CN): $\lambda_{max}$/nm (ε M$^{-1}$ cm$^{-1}$) 260 (1.3–10$^3$), 269 (1.5–10$^3$), 274 (1.5–10$^3$), 292 (sh, 5.1–10$^2$). FT-IR (ATR, v (cm$^{-1}$)) 3084(w), 3057 (w), 2990(w), 2928(w), 2893(w), 2820(w), 1607(m), 1599(m), 1570(w), 1485(s), 1471(w), 1454(m), 1445(s), 1424(w), 1377(w), 1335(w), 1322(w), 1296(w), 1272(s), 1258(w), 1157(w), 1109(s), 1051(s), 1019 (m), 997(m), 966(w), 926(w), 919(m), 793(m), 765(s), 758(s), 735(m), 685(w), 621(w), 591(w), 579(w), 542(w). MS (ESI) m/z 368.0738 [M]$^{+}$ and 781.1424 [M+HCO$_3$]$^{+}$. Elemental Analysis. Calcul. For C$_{35}$H$_{29}$Cl$_2$N$_2$O$_4$Zn$_2$MeOH: C = 47.93, H = 4.42, N = 8.38; found C = 48.27, H = 4.15, N = 8.66.

**X-ray Diffraction**: A colourless crystal of the composition [Zn$_2$(MeOH)$_2$](ClO$_4$)$_2$ was embedded in inert perfluoropolyalkylether (viscosity 1800 cSt; ABCR GmbH) and mounted using a Hampton Research Cryoloop. The crystal was then flash cooled to 100.0(1) K in a nitrogen gas stream and kept at this temperature during the experiment. The crystal structure was measured on a SuperNova diffractometer with Atlas S2 detector using a CuKα microfocus source. The measured data was processed with the CrysalisPro (v40.53) software package.[19] Using Olex2,[20] the structure was solved with the ShelX[21] structure solution program using Intrinsic Phasing and refined with the ShelXL[22] refinement package using Least Squares Minimization. All non-hydrogen atoms were refined anisotropically. Most hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters. The position of H2, which is connected to O2 of the methanol ligand, was observed from difference Fourier maps and refined. The crystal structure data has been deposited with the Cambridge Crystallographic Data Centre. CCDC 2036662 contains the supplementary crystallographic data for complex [Zn$_2$(MeOH)$_2$](ClO$_4$)$_2$. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Crystallographic and refinement data are summarized in Tables S1–S3 (see Supporting Information).

**Stopped-flow UV-vis measures**: The reaction between Zn$_2$L$_2$ (25 µM) and KO$_2$ (4 mM, 6 mM [18]-crown-6) has been monitored in dry DMSO by using a Biofrm S600 instrument, using syringes 1, 2, and 3, equipped with an Energetik LDL5 ENQ EQ-99-FC laser-driven light source and a J&M TIDAS diode array detector (integration time 0.5 ms, $\lambda = 180–724$ nm).

**UV-vis measures with CAN**: The reaction between Zn$_2$L$_2$ (100 µM) and 1 eq of CAN has been monitored in dry acetonitrile (every 60 s for 2 h) by using an Analytik Jena AG Spectroord 200 UV-VIS-NIR spectrometer.

**CV Experiments with KO$_2$**: The Zn$_2$L$_2$O$_4$$^{2-}$ complex has been obtained adding to a Zn$_2$L$_2$ (0.5 mM) solution in dry acetonitrile 250 µL (1 eq) of a KO$_2$/[18]-crown-6 dimethylformamide dry solution (10 mM) and measuring the CV after few second mixing.

**CSI-MS Experiments with KO$_2$**: The Zn$_2$L$_2$O$_4$$^{2-}$ complex has been obtained adding to a 0.1 mM solution of Zn$_2$L$_2$ in acetonitrile previously cooled down to -40°C. EPR Experiments with KO$_2$**: The Zn$_2$L$_2$O$_4$$^{2-}$ complex has been obtained adding to a 1 mM solution in dry DMSO 50 µL (5 eq) of a KO$_2$/[18]-crown-6 dry DMSO solution (10 mM) and immediately freezing the sample in liquid nitrogen.

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**Keywords**: superoxide · zinc complex · phenoxyl radical · Lewis acid · electron transfer

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