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Communication

Reactivity of Mn^{II} with Superoxide. Evidence for a $[Mn^{III}OO]^+$ Unit by Low-Temperature Spectroscopies

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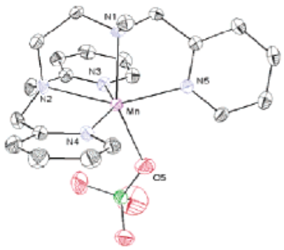
Manganese superoxide dismutase cycles between the Mn^{III} and Mn^{II} states to produce oxygen and hydrogen peroxide from superoxide. The formation of an adduct has been suggested, but its nature remains questionable because both $[Mn^{II}OO^-]$ and $[Mn^{III}OO^{2-}]$ redox states have been proposed. Study of the reactivity of superoxide with manganese complexes is of current interest. The reaction of $[(L)Mn^{II}]^{2+}$ [L = *N*-methyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)ethane-1,2-diamine] with potassium superoxide has been investigated at low temperature in an anhydrous solvent using various techniques. Upon the addition of ca. 2 equiv of potassium superoxide, the $[(L)Mn^{II}]^{2+}$ colorless solution turned blue and the UV–vis spectrum displayed a band at 590 nm (165 M^{-1} cm^{-1}) and a shoulder at 430 nm (100 M^{-1} cm^{-1}). Electrospray ionization mass spectrometry showed a peak ($m/z = 434.1$) assigned to $[(L)MnO_2]^+$. The X-band electron paramagnetic resonance spectrum parallel mode displayed a six-line signal separated by 6.6 mT and centered at 86 mT ($g = 8.1$). These results support the formation of an $[Mn^{III}OO]^+$ adduct.

Manganese superoxide dismutase (MnSOD) catalyzes the disproportionation of the harmful superoxide (O_2^-). This enzyme is involved in the cell protection against oxidative stress.[1,2] The MnSOD cycles between the Mn^{III} and Mn^{II} states to produce oxygen and hydrogen peroxide (H_2O_2). The study of the reactivity between superoxide and MnSOD or manganese model complexes is of current interest in order to provide a better description of the catalytic cycle. Adducts labeled $[MnO_2]^+$ have often been suggested in the literature,[3−6] but the assignment of the oxidation states of the Mn center and the oxygen ligand [manganese(III) peroxo or manganese(II) superoxo] is still controversial.[6,7] In this Communication, we present the characterization of an adduct resulting from the reaction between O_2^- and the $[(L)Mn^{II}]^{2+}$ complex (**1**) at low temperature [L = *N*-methyl-*N*,*N*′,*N*′-tris(2-pyridylmethyl)ethane-1,2-diamine]. X-band parallel-mode electron paramagnetic resonance (EPR) spectroscopy, UV−vis spectroscopy, and electrospray ionization mass spectrometry (ESI-MS) evidenced the formation of a monomeric manganese(III) peroxo species.



d(Mn-O5)= 2.262(4)
d(Mn-N1)= 2.293(3)
d(Mn-N2)= 2.232(3)
d(Mn-N3)= 2.242(3)
d(Mn-N4)= 2.232(3)
d(Mn-N5)= 2.232(3)
N5-Mn-O5= 81.47(13)
N5-Mn-N4= 112.63(12)
N5-Mn-N3= 100.62(12)
N5-Mn-N2= 151.72(11)
N5-Mn-N1= 73.75(11)

Figure 1. ORTEP drawing of **2** (30% thermal ellipsoids). Selected bond distances (Å) and angles (deg).

The pentadentate ligand L was chosen for this study based on the fact that it had been successfully used with iron to generate peroxo adducts.[8] The reaction of L with $[Mn^{II}(OH_2)_6](ClO_4)_2$ led to the crystallization of $[(L)Mn^{II}(ClO_4)](ClO_4)$ (**2**)(ClO_4) (Figure 1).[9] Figure 1 shows that the sixth position ($O-ClO_3$) remains widely accessible.

In the present study, the $[(L)Mn^{II}]^{2+}$ (**1**) species was generated in situ by mixing L and anhydrous $Mn^{II}(CF_3SO_3)_2$[10,11] in anhydrous acetonitrile (MeCN)/dimethyl sul-

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(9) Crystal data for **2**: colorless rectangular plate, triclinic, $P\bar{1}$, $a = 9.0624(10)$ Å, $b = 9.6172(12)$ Å, $c = 16.3580(19)$ Å, $a = 83.790(3)°$, $\beta = 86.694(3)°$, $\gamma = 62.736(3)°$, $V = 259.8(3)$ Å^3, 3059 reflections ($2.80° < \theta < 30.11°$), R (R_w) = 0.055 (0.137). CCDC 614162 contains the supplementary crystallographic data that can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_ request/cif.

10.1021/ic062063+ CCC: $37.00

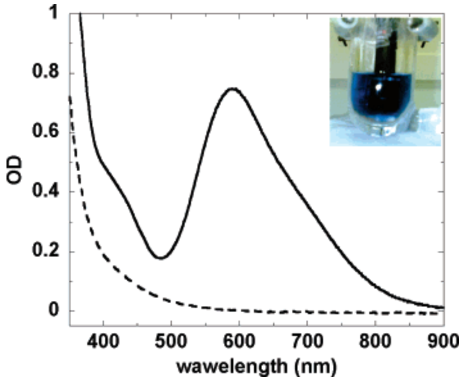

Figure 2. UV−vis spectrum of an anhydrous MeCN/DMSO (12 mL/300 μL) solution of **1** (5 mM) after the addition of ca. 2 equiv of KO_2 at −25 °C (solid line) and of the initial $[(L)Mn^{II}]^{2+}$ solution (dashed line). Inset: Picture of the blue solution in the double-jacket cell with the immersion probe (black part).

foxide (DMSO) (5 mM). Potassium superoxide (ca. 2 equiv) was added as a fine powder directly into this solution at −25 °C. The colorless solution slowly turned blue over half an hour. An UV−vis spectrum was recorded at −25 °C using a previously reported setup.[12] Figure 2 shows the final spectrum with a well-defined band at 590 nm and a shoulder at 430 nm. Assuming that 100% of Mn^{II} was converted, values of 165 and 100 M^{-1} cm^{-1} per Mn center were calculated for the extinction coefficients for $\lambda = 590$ and 430 nm, respectively. The blue species slowly decayed at room temperature over 30 min.

The UV−vis spectrum is reminiscent of that detected by stopped flow and previously reported in the literature in the case of the addition of KO_2 to a solution of $[Mn^{II}(EDTA)]$ (EDTA = ethylenediamminetetraacetate).[5] The appearance of a transient blue color has also been noticed for a manganese(II) complex derived from 1,4,7-triazacyclononane ligand upon H_2O_2 oxidation.[13] Moreover, crystals of a side-on manganese(III) peroxo complex have been obtained at low temperature by the addition of H_2O_2 on a manganese(III) complex.[14] This manganese(III) peroxo complex displayed a band at 583 nm ($\epsilon \approx 70$ M^{-1} cm^{-1}). In addition, Brunold et al. have reported calculated values at $\lambda \sim 645$ and 465 nm ($\epsilon \approx 450$ and 700 M^{-1} cm^{-1}) for the d−d transitions of a proposed side-on $(\eta^2\text{-}O_2)Mn^{3+}$SOD adduct known as the “dead-end complex.”[15]

The ESI-MS spectrum showed a peak at *m*/*z* 434.1 that supports the assignment of the blue species to a $[(L)MnO_2]^+$ complex ($C_{21}H_{25}N_5O_2Mn$).

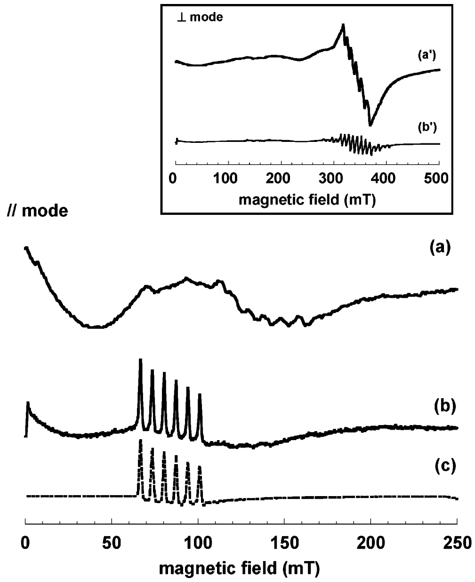

Figure 3. Parallel-mode detection EPR spectra of a solution of **1** in MeCN/DMSO (5 mM) (a) before the addition of KO_2 and (b) after the addition of ca. 2 equiv of KO_2 (solid line); XSophe-simulated spectrum with $D = -2.9$ cm^{-1}, $E/D = 0.075$, $g_z = 1.98$, $A_z = 0.00635$ cm^{-1} (dashed line). Inset: Perpendicular-mode detection EPR spectra of a solution of **1** in MeCN/DMSO (5 mM) (a′) before the addition of KO_2 and (b′) after the addition of ca. 2 equiv of KO_2. Recording conditions: two scans; microwave power, 2 mW; modulation amplitude, 0.5 mT; modulation frequency, 100 kHz; time constant, 40.96 ms; $T = 5$ K; perpendicular mode, $\nu = 9.63$ GHz; parallel mode, $\nu = 9.42$ GHz.

The blue solution was transferred into an EPR tube and frozen at liquid nitrogen. To provide further evidence for the electronic structure of the blue species, both perpendicular- and parallel-mode EPR spectra were recorded.[16]

The spectrum of **1** (initial solution) recorded in the perpendicular mode (inset Figure 3a′) displayed an intense resonance at 320 mT ($g = 2$), showing a six-line hyperfine structure with an average spacing of 8.5 mT and two less intense resonances at around 135 and 190 mT. These features are consistent with a distorted manganese(II) complex.[17,18] In the parallel mode, a broad signal was detected that displayed resonances from 7 to 180 mT (Figure 3a) with hyperfine structures separated by approximately 9 mT.

The X-band parallel-mode EPR spectrum of the blue solution recorded at 5 K is shown in Figure 3b. The signal consisted of six lines separated by 6.6 mT and centered at 86 mT ($g = 8.1$). It was checked that the growing of the 590 nm band correlates with the growing of the $g = 8.1$ six-line EPR signal (see Figure S1 in the Supporting Information). Simulation of the spectrum led to a $|D|$ value of 2.9 cm^{-1} (see Figure 3). The temperature dependence of the intensity indicated a negative value for D (Figure S2 in the Supporting Information). These features are characteristic of monomeric manganese(III) complexes ($S = 2$).[19−22] The

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signal detected in the perpendicular mode (inset Figure 3b′) is characteristic of the formation of a mixed-valent Mn^{III}-$Mn^{IV}(\mu\text{-}O)_2$ complex,[17] which only accounted for 8% of the Mn present in the solution.[23,24]

The above-reported experiments strengthen the occurrence of the putative $[MnO_2]^+$ adduct in the course of the reaction of O_2^- with manganese(II) complexes. The present parallel-mode EPR data unambiguously support a Mn^{III} oxidation state. So far, in the absence of vibrational spectroscopic data, the nature of the Mn−O bond (end-on or side-on) cannot be specified.[16]

We shall emphasize that for the first time a $[(L)Mn^{III}OO]^+$ species is detected by both parallel-mode EPR and UV−vis spectroscopy. Whereas the characterization of this type of intermediate is well documented in the literature in the case of iron and copper,[25−27] it is so far rare in manganese chemistry.

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Supporting Information Available: Experimental procedures for the synthesis of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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