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Mimicking Class Ib Mn_2 -Ribonucleotide Reductase: A Mn^II_2 Complex and Its Reaction with Superoxide

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Abstract

A fascinating discovery in the chemistry of ribonucleotide reductases (RNRs) has been the identification of a dimanganese (Mn_2) active site in class Ib RNRs that requires superoxide anion ($O_2^{\bullet-}$), rather than dioxygen (O_2), to access a high-valent Mn_2 oxidant. Complex **1** ($[Mn_2(O_2CCH_3)(N-Et-HPTB)](ClO_4)_2$, N-Et-HPTB=N,N,N',N'-tetrakis(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3-diaminopropane) was synthesised in high yield (90%). **1** was reacted with $O_2^{\bullet-}$ at $-40\text{ }^\circ\text{C}$ resulting in the formation of a metastable species (**2**). **2** displayed electronic absorption features ($\lambda_{max}=460, 610\text{ nm}$) typical of a Mn-peroxide species and a 29-line

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Conflict of interest

The authors declare no conflict of interest.

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EPR signal typical of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ entity. Mn K-edge X-ray absorption near-edge spectroscopy (XANES) suggested a formal oxidation state change of Mn^{II} in **1** to $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ for **2**. Electrospray ionisation mass spectrometry (ESI-MS) suggested **2** to be a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complex. **2** was capable of oxidizing ferrocene and weak O–H bonds upon activation with proton donors. Our findings provide support for the postulated mechanism of $\text{O}_2^{\bullet-}$ activation at class Ib Mn_2 RNRs.

Keywords

bioinorganic chemistry; dimanganese complexes; dioxygen/superoxide activation; oxidation reactions; ribonucleotide reductases

Ribonucleotide reductases (RNRs) are essential enzymes that convert ribonucleotides to their corresponding deoxyribonucleotides, providing the precursors required for DNA synthesis and repair in all organisms.^[1] Three different RNR classes (I, II, and III) have been reported, with class I further divided into subclasses Ia, Ib, and Ic. All three classes use different metallo-cofactors.^[2] In subclasses Ia and Ib, through O_2 -activation, the metallo-cofactor is postulated to oxidize a tyrosine group, forming a tyrosyl radical that mediates the generation of a cysteine (thiyl) radical. The thiyl radical in turn initiates nucleotide reduction.

Inspired by a recent study on class Ib dimanganese (Mn_2) RNRs,^[3] we became interested in the role of superoxide anion ($\text{O}_2^{\bullet-}$, Scheme 1). Stubbe and colleagues demonstrated that the Mn_2 cofactor showed no reaction with O_2 .^[3d] However, in the presence of a flavodoxin protein (NrdI_{hq} , flavodoxin hydroquinone) O_2 was reduced to $\text{O}_2^{\bullet-}$. The $\text{O}_2^{\bullet-}$ was proposed to react with the Mn^{II}_2 core yielding a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ peroxide entity.^[3a] Subsequent cleavage of the O–O bond to generate a $\text{Mn}^{\text{III}}(\mu\text{-OH})(\mu\text{-O})\text{Mn}^{\text{IV}}$ species that oxidizes tyrosine was also proposed.^[3d] EPR spectroscopy supported the formation of a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ intermediate from the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, but no insight into the initial species was obtained.^[3d]

Synthetic Mn_2 model complexes that mimic some of the intermediates in Scheme 1 have been prepared.^[4] A series of mononuclear Mn^{III} -peroxide and hydroperoxide species have been reported.^[5] These species were generated by treating mononuclear Mn^{II} precursors with a variety of oxidants including O_2 ,^[5g,k] $\text{O}_2^{\bullet-}$,^[5d,i] and H_2O_2 .^[5b,c,e,f,h-j] Model compounds that feature bis- μ -oxo- $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ cores that could serve as potential mimics of the $\text{Mn}^{\text{III}}(\mu\text{-OH})(\mu\text{-O})\text{Mn}^{\text{IV}}$ species in RNRs have also been reported.^[4a, 6, 5g] These high-valent complexes formed upon treatment of mononuclear Mn^{II} complexes with O_2 or H_2O_2 .^[4a, 6c] Jackson and co-workers recently reported the formation of a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species using $\text{O}_2^{\bullet-}$ as an oxidant from mononuclear Mn^{II} precursors.^[7] Initially, a mononuclear Mn^{III} -peroxide adduct was formed, which subsequently reacted with the mononuclear Mn^{II} precursor forming the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. To the best of our knowledge, however, a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complex has not been previously reported. Furthermore, no investigations into the reaction between Mn^{II}_2 complexes and $\text{O}_2^{\bullet-}$ have been reported. In order to probe the above mechanistic postulates, we explore herein the interaction between a synthetic Mn^{II}_2 complex and $\text{O}_2^{\bullet-}$.

$[\text{Mn}_2(\text{O}_2\text{CCH}_3)(\text{N-Et-HPTB})](\text{ClO}_4)_2$ (**1**, N-Et-HPTB=*N,N,N',N'*-tetrakis(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3-diaminopropane^[9]) was synthesised using a slight modification of the procedure reported for the preparation of $[\text{Mn}_2(\text{O}_2\text{CCH}_3)(\text{HPTB})](\text{ClO}_4)_2$ (**1'**, HPTB=*N,N,N',N'*-tetrakis(2-(benzimidazolyl))-2-hydroxy-1,3-diaminopropane).^[10] Elemental analysis and matrix-assisted laser-desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry confirmed the elemental composition of **1**. The electron paramagnetic resonance (EPR) spectra of complexes **1** and previously reported **1'** showed very similar signals ($g=2.0$; Figures S1 and S2, Supporting Information). The obtained EPR signal was assigned to axially distorted Mn^{II} sites, as described by Boelrijk et al. for complex **1'**.^[11]

Crystals of **1** suitable for X-ray diffraction measurements were grown from CH_3CN by diethyl ether (Et_2O) vapour diffusion. **1** was found to consist of two five-coordinate Mn^{II} atoms both with a distorted trigonal-bipyramidal geometry (Figure 1). The average Mn– N_{amine} and Mn– N_{benz} bond lengths of **1** were shorter than those of **1'**,^[10] presumably as a result of the higher basicity of the alkylated ligand (N-Et-HPTB) in **1**. **1** displayed a Mn··Mn separation of 3.6 Å versus 3.5 Å for **1'**. Interestingly, the $\text{Mn}^{2+}\cdots\text{Mn}^{2+}$ distance in the X-ray crystal structures reported for class Ib Mn_2 RNRs from *E. coli* was 3.7 Å,^[3a] from *B. subtilis* 3.9 Å, while from *C. ammoniagenes* a $\text{Mn}^{3+}\cdots\text{Mn}^{3+}$ separation of 3.3 Å was measured.^[3c] For all three structures of Mn_2 RNRs the Mn active sites displayed either at least one vacant site on the metal or a labile ligand that could be the location of $\text{O}_2^{\bullet-}$ binding. Importantly, in **1** the Mn ions were similarly not coordinatively saturated. The structural data obtained for **1** thus compared favourably with these enzymes, suggesting that **1** was a good structural mimic for class Ib Mn_2 RNRs.

To a CH_3CN solution containing complex **1** (1 mM, 2 mL, cooled to -40°C) was added a *N,N*-dimethylformamide (DMF, 0.3 mL) solution containing 3 equiv KO_2 (20 mM in DMF) and 18-crown-6 (59 mM in DMF) (Scheme 2). An immediate reaction occurred (complete in 35 s) resulting in the formation of a new species (**2**), as evidenced by electronic absorption spectroscopy (Figure 2). The electronic absorption spectrum of **2** displayed low-intensity features at $\lambda_{\text{max}}=460$ and 610 nm (Figure 2), whereas **1** displayed limited absorptivity above 300 nm. At higher concentrations of **1**, lower yields of **2** were obtained (Supporting Information, Figure S3). This is presumably as a result of intermolecular interactions preventing the formation or accelerating the decay of **2** at higher concentrations of **1**.^[12]

We noted that the electronic absorption features for **2** were characteristic of Mn^{III} -peroxide complexes.^[13,7] Previously reported Mn^{III} -peroxide complexes $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{TMC})]^+$ (TMC=1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane), $[\text{Mn}^{\text{III}}(\text{O}_2)(13\text{-TMC})]^+$ (13-TMC=1,4,7,10-tetramethyl-1,4,7,10-tetraazacyclotridecane), and $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{Tp}^{\text{iPr}})]^+$ (Tp^{iPr} =tris(3,5-diisopropylpyrazolyl)borate), all exhibited an absorption band at around 460 nm attributed to peroxide-to-metal charge transfer^[14] and a broader band in the 560–620 nm range, derived from $d-d$ transitions.^[14] In addition, Mn^{III} -peroxides supported by other polydentate amine ligands exhibited a prominent band at $\lambda_{\text{max}}=430\text{--}445$ nm and a weaker band at $\lambda_{\text{max}}=590\text{--}610$ nm.^[15] We therefore concluded that **2** contained a Mn-peroxide core.

Cold injection electrospray ionisation mass spectrometry (ESI-MS) experiments on a just-thawed CH₃CN solution of **2** revealed ion peaks consistent with **2** being a Mn^{II}Mn^{III} peroxide complex. A mass peak ($m/z=431.54$) consistent with the formulation of the di-cation [Mn₂(O₂)(N-Et-HPTB)]²⁺ (Figure 3, left) was obtained. When **2** was prepared with K¹⁸O₂, cold injection ESI-MS of the ¹⁸O-labelled **2** resulted in a mass peak at $m/z=433.48$, a mass that can be ascribed to the di-cation [Mn₂(¹⁸O₂)(N-Et-HPTB)]²⁺ (Figure 3, right). These results led us to define **2** as a Mn^{II}Mn^{III}-peroxide complex derived from O₂^{•-}.

Raman and infrared spectroscopy studies on **2** were attempted but failed to provide any insight. In contrast, Kovacs and co-workers were successful in observing the ν_{O-O} and the ν_{Mn-O} of a thiolato-Mn^{III}₂-peroxide complex by resonance Raman spectroscopy,^[5k] while Nam and Solomon reported the ν_{O-O} of a Mn^{IV}-peroxo complex.^[16] These are the only two examples of a vibrational analysis of Mn-dioxygen species.^[5e,h,j]

2 displayed a 29-line EPR signal at 2 K (Figure 4), resembling those observed for several Mn^{II}Mn^{III} complexes.^[17] This group includes a recent example reported by Borovik and co-workers to have a Mn^{II}-(μ -OH)-Mn^{III} core.^[18] The signal is typical of a Mn^{II}Mn^{III} species with an effective $S=1/2$ ground state ($g\approx 1.96$).^[17d] The observation of multiple lines derives from hyperfine interactions with the two nonequivalent Mn ions. The yield of **2**, as determined by EPR integration, was circa 80% (see the Supporting Information, Figure S4). The broad signal at circa 1000 G has been seen in previously reported Mn^{II}Mn^{III} complexes and was assigned by those groups to an $S=3/2$ spin state.^[17b,c,19] The similarities in EPR data between the previously reported Mn^{II}Mn^{III} complexes and that obtained for **2** lead us to assign **2** as a Mn^{II}Mn^{III} complex.

In order to further understand the Mn oxidation states in **2**, we performed Mn K-edge X-ray absorption near-edge spectroscopy (XANES) on frozen solutions of **1** and **2**. The first inflection of the rising edge was found to be 6547.7 eV for **1** (Figure 5), consistent with assignment to the Mn^{II} state. **2** exhibited an increase in edge energy of circa 1 eV, to 6548.7 eV, relative to **1**. Notably, the 1s-to-3d pre-edge transition was found at identical energy (6540.4 eV) for both complexes. Previous reports suggested that each integer change in Mn oxidation state was accompanied by a 2–4 eV blue-shift in Mn K-edge energy, while the pre-edge energies were largely invariant for the Mn^{II} and Mn^{III} states.^[20] Our observation of a 1-eV blue-shift in edge energy and unchanged pre-edge energy is consistent with a half-integer change in average Mn valence, and the assignment of **2** as a Mn^{II}Mn^{III} complex. Extended X-ray absorption fine structure (EXAFS) measurements were not performed because we were unable to obtain **2** in a sufficiently high concentration (optimal yield obtained at 1 mM of **1**) to allow accurate EXAFS analyses.

2 decayed very slowly at low temperatures ($-40\text{ }^{\circ}\text{C}$, $t_{1/2}\approx 4\text{ h}$), but it decayed within 4 min upon warming to room temperature. At $-40\text{ }^{\circ}\text{C}$ the electronic absorption spectrum of **2** was unaffected by the addition of triphenylphosphine (PPh₃), cyclohexene, or substrates containing weak C–H bonds (all added in 60-fold excess, including TEMPO-H (2,2,6,6-tetramethyl-piperidine-1-ol, C–H bond dissociation energy (BDE)=70.6 kcalmol⁻¹),^[21] 1-methyl-1,4-cyclohexadiene (BDE_{C-H}=77 kcalmol⁻¹),^[22] and dihydroanthracene (BDE_{C-H}=78 kcalmol⁻¹)^[23]). The lack of any reaction with this group of substrates

demonstrated that **2** was not a capable electrophilic oxidant. Furthermore, **2** was unreactive towards aldehydes, indicating it was also a poor nucleophilic oxidant.^[5j, 14, 15, 24] **2** was also not reduced by ferrocene. We thus concluded that the Mn^{II}Mn^{III}-peroxide unit in **2** was unreactive at -40°C.

2 reacted readily with proton donors (*para*-toluenesulfonic acid (TsOH), HBF₄) resulting in the immediate disappearance/bleaching of the electronic absorption features associated with **2** (Supporting Information, Figure S5). The addition of HBF₄ to **2** in the presence of ferrocene likewise caused the immediate decay of the features associated with **2**, alongside the appearance of electronic absorption features attributed to the ferrocenium cation (Figure 6). The yield of ferrocenium formed was calculated to be circa 20% with respect to the concentration of **1** in the initial reaction mixture through electronic absorption spectroscopy. Ferrocene was not oxidised under the same reaction conditions in the absence of **1** or **2**, demonstrating that H⁺-donor mediated activation of **2** yielded a capable Mn oxidant. Furthermore, ferrocene did not react with KO₂ or H₂O₂ under the same conditions. Similarly, when **2** was reacted with HBF₄ in the presence of TEMPO-H we identified TEMPO radical as a product of the reaction using EPR spectroscopy (Figure 6 and the Supporting Information, Figures S5 and S6). Although TEMPO-H did not react with KO₂, it did react with H₂O₂ under the same reaction conditions, suggesting that the oxidation of TEMPO-H could be caused by either a Mn₂ oxidant or released H₂O₂. We also observed the formation of free Mn^{II} ions, as evidenced by a 6-line EPR signal, which can be ascribed to de-complexation, although the mechanism for this event is as yet not clear.

We surmise that the H⁺-donor activated the Mn^{II}Mn^{III} peroxide core of **2**, yielding an oxidant that was capable of electron transfer (ferrocene) and hydrogen atom transfer (TEMPO-H). This mimics the postulated role of proton donors in class Ib Mn₂ RNRs, in which protonation of the metal-bound peroxide is postulated to precede the formation of the tyrosine-oxidising high-valent Mn₂ oxidant.^[3d]

We have provided experimental insight into Stubbe's proposed mechanism of O₂^{•-} activation at Mn₂ RNRs. The Mn₂ complex **1** reacted with O₂^{•-}, yielding a Mn^{II}Mn^{III} peroxide complex (**2**). **2** was further shown to be activated by proton donors, yielding an unidentified species capable of electron transfer and oxidative activation of O-H bonds, presumably through hydrogen atom transfer. This provides experimental insight into the postulated biochemistry of Mn₂ RNRs, in which both a proton and O₂^{•-} are postulated to be required to access a high-valent oxidant via a Mn^{II}Mn^{III} peroxide intermediate. Work continues in our labs to probe this mechanism further and trap the putative high-valent oxidant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. ORTEP structure of **1**. Hydrogen atoms and perchlorate anions have been omitted for clarity. Ellipsoids are shown at 50% probability. Selected bond distances [Å]: Mn1–Mn2 3.6; Mn1–O1 2.05, Mn1–O2 2.08, Mn1–N13 2.42, Mn1–N1 2.12, Mn1–N14 2.13, Mn2–O1 2.04, Mn2–O3 2.07, Mn2–N38 2.41, Mn2–N26 2.13, Mn2–N39 2.13.

Figure 2.
Electronic absorption spectra of **1** (black trace, 1 mM) and **2** (grey trace, from **1** (1 mM) +KO₂) at -40 °C in CH₃CN.

Figure 3. ESI-MS spectra of **2** prepared using $K^{16}O_2$ (left) and $K^{18}O_2$ (right). Insets: Simulated mass spectra.

Figure 4. Perpendicular mode EPR spectrum of **2** at 2 K (obtained from the reaction of 1 mM **1** and KO_2 in CH_3CN) (9.64 GHz, 0.2 mW microwave power, 1mM of **1**).

Figure 5. Normalized XANES spectra of **1** (—) and **2** (-----). The inset shows an expansion of the pre-edge region.

Figure 6.

Electronic absorption spectra (left) showing formation of ferrocenium (Fc^+ , $\lambda_{\text{max}}=620$ nm, grey trace) from the reaction of **2** (black trace) with H^+ in the presence of ferrocene; normalised EPR spectrum (right) exhibiting the reference TEMPO radical (black trace) and TEMPO radical formation (grey trace) from the reaction of **2** with H^+ in the presence of TEMPO-H.

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Scheme 2.

Reaction of **1** with $\text{O}_2^{\bullet-}$ forming a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complex, **2**.