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 ribonucleotide reductase’s (RNRs) chemistry has been the identification of a dinuclear manganese (Mn$_2$) active site in class Ib RNRs that requires superoxide anion (O$_2^•$), rather than dioxygen (O$_2$), to access a high-valent Mn$_2$ oxidant. Complex 1 [(Mn$_2$(O$_2$CCH$_3$)(N$_2$E-HPTB))(ClO$_4$)$_2$, N$_2$E-HPTB = N,N,N,N'-tetrais(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3-diaminopropane] was synthesised in high yield (90 %). 1 was reacted with O$_2$ at -40 °C resulting in the formation of a metastable species (2). 2 displayed electronic absorption features ($\lambda_{max} =$ 460, 610 nm) typical of a Mn-peroxide species, and a 29 line EPR signal typical of a Mn$_2$Mn$^{III}$ entity. Mn K-edge X-ray absorption near-edge spectroscopy (XANES) suggested a formal oxidation state change of Mn$_2^{IV}$ to Mn$_2$Mn$^{III}$ for 2. Electrospray ionisation mass spectrometry (ESI-MS) confirmed the presence of a flavodoxin protein (NrdI$_{sq}$, flavodoxin hydroquinone) O$_2$ was reduced to O$_2^•$ by the NrdI$_{sq}$ complex and O$_2^•$ was proposed to react with the Mn$_2$ core yielding a Mn$_2$Mn$^{IV}$-peroxide entity. Subsequent cleavage of the O–O bond to generate a Mn$_2$Mn$^{III}$ species that oxidizes tyrosine was also proposed. EPR spectroscopy supported the formation of a Mn$_2$Mn$^{III}$ intermediate, but no insight into the initial species was obtained.

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 species have been reported.$^5$ Model compounds that feature bis-µ-oxo-Mn$^{III}$Mn$^{IV}$ cores that could serve as potential mimics of the Mn$^{III}$-(µ-OH)-(µ-O)Mn$^{IV}$ species have also been reported.$^6$ Jackson and co-workers recently reported the formation of a Mn$^{III}$Mn$^{IV}$ species using O$_2^•$ as an oxidant from mononuclear Mn$^{III}$ precursors.$^7$ To the best of our knowledge, however, a Mn$_2$Mn$^{III}$-peroxide complex has not been previously reported. Furthermore, no investigations into the reaction between Mn$_2$ complexes and O$_2^•$ have been reported. In order to probe the above mechanistic postulates, herein we explore the interaction between a synthetic Mn$_2$Mn$^{III}$ complex and O$_2^•$.

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, III) have been reported, with class I further divided into sub-classes Ia, Ib, and Ic. All three classes use different metallo-cofactors.$^{[2]}$ In class Ia and Ib, through O$_2$-activation, the metallo-cofactor is postulated to oxidize a tyrosine group, forming a tyrosyl radical which mediates the generation of a cysteine (thiol) radical. The thiol 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 (O$_2^•$; Scheme 1). Stubbe and colleagues demonstrated that the Mn$_2$ cofactor showed no reaction with O$_2$.$^{[2a]}$ However, in the presence of a flavodoxin protein (NrdI$_{sq}$, flavodoxin hydroquinone) O$_2$ was reduced to O$_2^•$.$^{[3d]}$ The O$_2^•$ was proposed to react with the Mn$_2$ core yielding a Mn$_2$Mn$^{IV}$-peroxide entity.$^{[3a]}$ The Mn$_2$Mn$^{IV}$-peroxide entity in turn initiates forming a tyrosyl radical which mediates the generation of a Mn$^{III}$-peroxide entity.$^{[3a]}$ The Mn$_2$Mn$^{III}$-peroxide entity oxidizes tyrosine to generate a Mn$^{IV}$-peroxide entity.$^{[3a]}$ The Mn$_2$Mn$^{IV}$-peroxide entity oxidizes tyrosine to generate a Mn$_2$Mn$^{IV}$-peroxide entity.$^{[3a]}$ The Mn$_2$Mn$^{IV}$-peroxide entity oxidizes tyrosine to generate a Mn$_2$Mn$^{IV}$-peroxide entity.$^{[3a]}$

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Scheme 1. Proposed catalytic cycle for class Ib Mn$_2$ RNRs in B. subtilis (NrdI$_{sq}$ = Flavodoxin hydroquinone, NrdI$_{sq}$ = Flavodoxin semiquinone).$^{[2a, 3a]}$

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[Mn$_2$(O$_2$CCH$_3$)(N$_2$E-HPTB)](ClO$_4$)$_2$ (1, N$_2$E-HPTB = N,N,N,N'-tetrais(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3-diaminopropane) was synthesised using a slight modification of the procedure reported for the preparation of [Mn$_2$(O$_2$CCH$_3$)(HPTB)](ClO$_4$)$_2$ (1', HPTB = N,N,N',N'-tetrais(2-(benzimidazolyl))-2-hydroxy-1,3-diaminopropane).$^{[5]}$ 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, Figure S1-S2, supporting information).
The obtained EPR signal was assigned to axially distorted Mn
sites, as described by Boelrijk et al. for complex 1.[11]

Crystals of 1 suitable for X-ray diffraction measurements were grown from acetonitrile (CH₃CN) by diethyl ether (Et₂O)
vapour diffusion. 1 was found to consist of two five-coordinate
Mn⁶ atoms both with a distorted trigonal-bipyramidal geometry
(Figure 1). The average Mn-Namine and Mn-Nbase bond lengths of 1 were shorter than those of 1',[10] presumably as a result of the higher basicity of the alkylation ligand (N-Et-HPTB) in 1. 1' displayed a Mn–Mn separation of 3.5 Å versus 3.6 Å for 1. Interestingly, the Mn²⁺–Mn³⁺–Mn³⁺ distance in the X-ray crystal structure reported for complex Ib Mn₂ RNRS from E. coli was 3.7 Å.[3a] From B. subtilis the Mn active sites displayed either at least one vacant site on the metal, or a labile ligand, presumably the location of O₂²⁻ binding. Importantly in 1, the Mn ions were similarly not coordinatively saturated. The structural data obtained for 1 thus compares favourably with these enzymes suggesting that 1 is a good structural mimic for class Ib Mn₂ RNRS.

To a CH₃CN solution containing complex 1 (1 mM, 2 mL) cooled to -40 °C was added an N,N-dimethylformamide (DMF, 0.3 mL) solution containingKO₂ (20 mM) and 18-crown-6 (59 mM) (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 λmax = 460 and 610 nm (Figure 2), whereas 1 displayed limited absorbivity above 300 nm. At higher concentrations of 1, lower yields of 2 were obtained (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]

[Scheme 2. Reaction of 1 with O₂²⁻ forming a Mn⁶/Mn³⁺-peroxide complex, 2.]

We noted that the electronic absorption features for 2 were characteristic of Mn⁶-peroxide complexes.[13, 7] Previously reported Mn⁶-peroxide complexes [Mn⁶(O₂)(TMC)]²⁺, (TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazaacyclotetradecane), [Mn⁶(O₂)(13-TMC)]²⁺, (13-TMC = 1,4,7,10-tetramethyl-1,4,7,10-tetraazaacyclotridecane) and [Mn⁶(µ-O)(TPd²⁺)]²⁺ (TPd²⁺ = tris(3,5-diisopropylpyrazolyl)borate) all exhibited an absorption band 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, Mn⁶-peroxides supported by other polydentate amine ligands exhibited a prominent band at λmax = 430-445 nm and a weaker band at λmax = 590-610 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 Mn⁶/Mn³⁺-peroxide complex. A mass peak (m/z = 431.54) consistent with the formulation of the di-cation [Mn₂(µ-O)(N-Et-HPTB)]²⁺ (Figure 3 right) 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, left). These results led us to define 2 as a Mn⁶/Mn³⁺-peroxide complex derived from O₂²⁻.

[Figure 3. ESI-MS spectra of 2 prepared using KO₂ (left) and K¹⁸O₂ (right). Insets: simulated mass spectra.]

Raman and infra-red spectroscopy studies on 2 were attempted, but failed to provide any insight. Kovacs and co-workers have successfully determined the νO₂ and the νO₂=O of a thiolato-Mn⁶³⁺-peroxide complex by resonance Raman spectroscopy.[5a]

However, this is the only successful example of a vibrational analysis of Mn-dioxoygen species.[5a, h, i]

2 displayed a 29-line EPR signal at 2 K (Figure 4), resembling those observed for several Mn⁶/Mn³⁺ complexes.[16] This group includes a recent example reported by Borovik and co-workers to have a Mn⁶⁺-(µ-OH)-Mn³⁺ core.[17] The signal is
typical of a Mn\textsuperscript{II}Mn\textsuperscript{III} species with an effective S = \textfrac{1}{2} ground state (g \approx 1.96).\textsuperscript{116} The observation of multiple lines derives from hyperfine interactions with the two non-equivalent Mn ions. The yield of 2, as determined by EPR integration, was \textasciitilde 80% (see supporting information, Figure S4). The broad signal at \textasciitilde 1000 G has been seen in previously reported Mn\textsuperscript{II}Mn\textsuperscript{III} complexes.\textsuperscript{116, 116c, 19} The similarities in EPR data between the previously reported Mn\textsuperscript{II}Mn\textsuperscript{III} complexes and that obtained for 2 lead us to assign 2 as a Mn\textsuperscript{II}Mn\textsuperscript{III} complex.

Figure 4. Perpendicular mode EPR spectrum of 2 at 2 K (9.64 GHz, 0.2 mW microwave power).

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\textsuperscript{II} state. 2 exhibited an increase in edge energy of \textasciitilde 1 eV, to 6548.7 eV, relative to 1. Notably, the 1s-2d pre-edge transition was found at identical energy (6540.4 eV) for both complexes. Previous reports suggest that every 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\textsuperscript{II} and Mn\textsuperscript{III} states.\textsuperscript{19} 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 assignment of 2 as a Mn\textsuperscript{II}Mn\textsuperscript{III} complex. Extended X-ray absorption fine structure (EXAFS) measurements were not performed because we were unable to obtain 2 in sufficiently high concentration (optimal yield obtained at 1 mM of 1) to allow accurate EXAFS analyses.

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

Efforts to model the structural and electronic properties of 2 using DFT methods are ongoing in our labs, and will be communicated in a later manuscript.

2 was stable at low temperatures (\textasciitilde 40 °C, \textasciitilde 04 hours) but it decayed within 4 minutes upon warming to room temperature. At \textasciitilde 40 °C the electronic absorption spectrum of 2 was unaffected by the addition of triphenylphosphine (PPh\textsubscript{3}), cyclohexene, or substrates containing weak C–H bonds (all added in 60-fold excess, including TEMPO–H \textleft(2,2,6,6-tetramethyl-piperidine-1-ol, C–H bond dissociation energy (BDE) = 70.6 kcal/mol\right),\textsuperscript{20} 1-methyl-1,4-cyclohexadiene (BDE\textsubscript{C=C} = 77 kcal/mol),\textsuperscript{21} and dihydroanthracene (BDE\textsubscript{C=C} = 78 kcal/mol)).\textsuperscript{22} 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.\textsuperscript{15} 2 was also unreactive towards ferrocene. We thus concluded that the Mn\textsuperscript{II}Mn\textsuperscript{III}-peroxide unit in 2 was stable and unreactive at \textasciitilde 40 °C.

2 reacted readily with proton donors (para-toluenesulfonic acid (TsOH), HBF\textsubscript{4}) resulting in the immediate disappearance/bleaching of the electronic absorption features associated with 2 (Figure S5). The addition of HBF\textsubscript{4} 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 \textasciitilde 20% with respect to the concentration of 1 in the initial reaction mixture through electronic absorption spectroscopy. Similarly, when 2 was reacted with HBF\textsubscript{4} in the presence of TEMPO–H we identified TEMPO radical as a product of the reaction using EPR spectroscopy (Figures 6, S5, S6). We also observed the formation of free Mn\textsuperscript{II} ions, as evidenced by a 6-line EPR signal, which can be ascribed to decomposition, although the mechanism of this event is as not yet clear.

We surmise that the proton donor activated the Mn\textsuperscript{II}Mn\textsuperscript{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 Mn\textsubscript{2} RNRs, where protonation of the metal-bound peroxide is postulated to precede formation of the tyrosine-oxidising high-valent Mn\textsubscript{2} oxidant.\textsuperscript{23}

We have provided experimental insight into Stubbe’s proposed mechanism of superoxide activation at Mn\textsubscript{2} RNRs. The Mn\textsubscript{2} complex 1 reacted with O\textsuperscript{2−}, yielding a Mn\textsuperscript{II}Mn\textsuperscript{III}-peroxide complex (2). 2 was further shown to be activated by proton donors, yielding an unidentified species capable of oxidative...
activation of O–H bonds, presumably through hydrogen atom transfer. This provides experimental insight into the postulated biochemistry of Mn₃RNRs, where both a proton and O₂• are postulated to be required to access a high-valent oxidant via a Mn₃Mn•••O₃-peroxide intermediate. Work continues in our labs to probe this mechanism further and trap the putative high-valent oxidant.

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Superoxide activation: We mimicked the chemistry of Class Ib Mn$_2$ ribonucleotide reductases by probing the reaction between a synthetic Mn$_2$$^{III}$ complex and superoxide. The reaction yielded a Mn$_2$$^{III}$/peroxide species. Proton donors activated the Mn$_2$$^{III}$/peroxide core facilitating electron transfer and hydrogen atom transfer reactivity. These observations provide experimental support for the postulated mechanism in Class Ib Mn$_2$-ribonucleotide reductases.