Peroxynitrite-Induced DNA Strand Scission Mediated by a Manganese Porphyrin

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Peroxynitrite ($ONOO^-$) is a potent oxidant[1] generated by the reaction between nitric oxide ($NO^•$) and superoxide ion ($O_2^{•-}$).[2] Thus, the macrophage immune response[3] or pathological conditions such as endotoxic shock and ischemia/reperfusion, which raise the concentrations of both $NO^•$ and $O_2^{•-}$, could generate significant levels of $ONOO^-$ *in vivo*.[4] Peroxynitrite and its conjugate acid are capable of nitrating tyrosine residues in proteins[5] and oxidizing DNA,[6] lipids,[7] sulfhydryls,[8] and methionine.[9] Hence, the toxicity of $ONOO^-$ has been considered in light of this reactivity.[10] Oxidative damage in cells has been linked to the metal ion-catalyzed generation of free radicals and peroxides.[11] Thus, we have examined the metal-mediated activation of peroxynitrite toward biological targets. Described here is evidence that a manganese porphyrin−peroxynitrite system generates reactive metal−oxo species which potentiate oxidative cleavage of plasmid DNA and catalyze the nitration of phenols.

We find that peroxynitrite[12] reacts rapidly and efficiently with MnTMPyP[13] under physiological conditions to generate an oxomanganese intermediate (Figure 1). A 1:1 stoichiometry is observed for the reaction, even though the half-life of $ONOO^-$ is <3 s under these conditions.[14] The high-valent species decayed back to the starting Mn(III) porphyrin with clear isosbestic behavior.[15] The intermediate observed was identified to be an $oxoMn^{(IV)}$ TMPyP species (**2**).[16] The spectroscopically

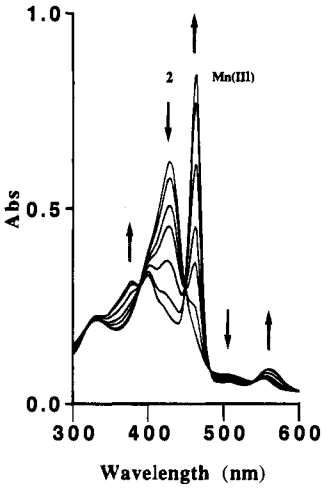

Figure 1. Reversion of oxoMn(IV) (**2**) (428 nm) to MnTMPyP (462 nm) (0−600 s). OxoMn(IV) was generated by a stoichiometric reaction between MnTMPyP (10 μM) and $ONOO^-$ (1 equiv) in 50 mM Tris (pH 7.4).

similar $oxoMn^{(V)}$ intermediate (**1**) has a very short lifetime under these conditions.[17]

Peroxynitrite in the presence of MnTMPyP caused extensive strand scission of plasmid DNA.[18a] Formation of the same oxoMn(IV) chromophore was observed under these conditions. DNA cleavage by $ONOO^-$ alone has been reported at much higher doses, but, notably, this cleavage was quenched in Tris buffer.[6] As can be seen in Figure 2, a dose-dependent increase of plasmid DNA cleavage was observed in lanes containing $ONOO^-$ *and* MnTMPyP, even in the presence of Tris buffer.[18b] Significantly, solutions depleted in $ONOO^-$ by spontaneous decay (10 min) failed to cleave DNA in the presence of the metalloporphyrin. Catalase concentrations up to 25 μg/mL had no effect on the $ONOO^-$−MnTMPyP-mediated DNA scission, while 2.5 μg/mL catalase was sufficient to afford complete protection from oxidative cleavage by H_2O_2 and MnTMPyP.[18c] The DNA cleavage observed is therefore attributed to the activation of $ONOO^-$ by MnTMPyP.[20]

We compared the MnTMPyP-catalyzed cleavage of pBR322 plasmids by peroxynitrite to those mediated by H_2O_2 and peroxymonosulfate (HSO_5^-).[20] HPLC profiles of reaction mixtures of MnTMPyP−HSO_5^- and MnTMPyP−$ONOO^-$ mediated cleavage of calf thymus DNA were identical, indicative of oxidative cleavage. Under conditions that accounted

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(18) (a) The Fe, Co, and Cu derivatives of TMPyP were also examined, but only FeTMPyP showed cleavage activity. (b) As expected, there was significantly more DNA cleavage in phosphate buffer (SSBs > 40). (c) While the $ONOO^-$ (25 μM) reaction was quenched after 2 min, the H_2O_2 (100 μM) reaction was incubated for 30 min.

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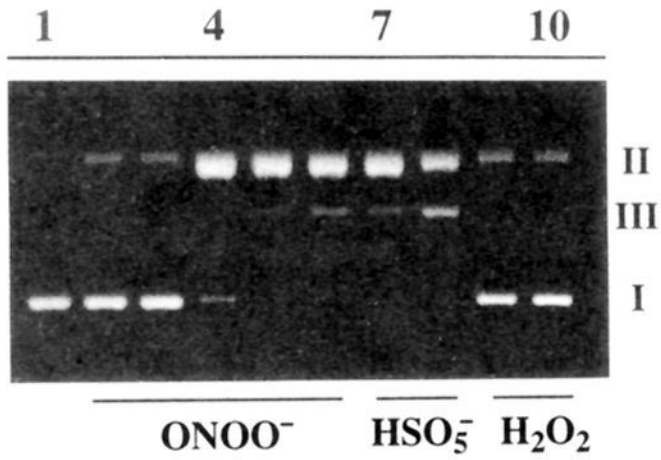
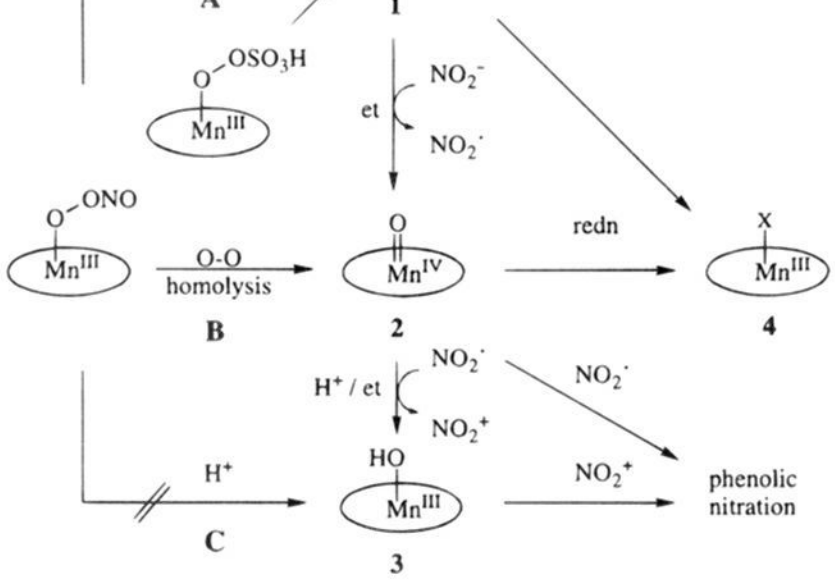


Figure 2. Comparison of plasmid DNA cleavage catalyzed by MnTMPyP−$ONOO^-$ to cleavage by MnTMPyP−H_2O_2 and MnTMPyP−HSO_5^-. pBR322 is 40 μM in each lane, while MnTMPyP is 500 nM in lanes 3−10. Reactions were initiated by the addition of the respective oxidants and quenched with Hepes (100 mM) after 25 s. Other details as before.[20b] In brackets after lane descriptions are single strand breaks (SSBs) calculated as described elsewhere.[19] Lane 1: pBR 322 (40 μM bp) control. Lane 2: $ONOO^-$ (100 μM) control (without MnTMPyP) [0.1]. Lane 3: depleted $ONOO^-$ (100 μM) [0.1]. Lane 4: $ONOO^-$ (25 μM) [5.0]. Lane 5: $ONOO^-$ (50 μM) [9.6]. Lane 6: $ONOO^-$ (100 μM) [11.1]. Lane 7: HSO_5^- (10 μM) [9.7]. Lane 8: HSO_5^- (25 μM) [14.5]. Lane 9: H_2O_2 (500 μM) [0.2]. Lane 10: H_2O_2 (1000 μM) [0.2].

Scheme 1



for $ONOO^-$ free decay,[14] the reactivity observed for $ONOO^-$ was similar to that of HSO_5^- and at least 2 orders of magnitude greater than that of H_2O_2 (Figure 2). The cleavage efficiency of the MnTMPyP−HSO_5^- system has been attributed to high-valent oxoMn species.[20a] The observation of the oxoMn(IV) species **2** (Figure 1) and the high reactivity of $ONOO^-$ suggest a similar mechanism. Moreover, *inhibition* of DNA cleavage resulted from the addition of NO_2^- to these reactions, indicating that NO_2^- was in active competition with the DNA for the reactive oxoMn species.[21] As summarized in Scheme 1, high valent oxoMn species can be generated by either an O−O bond heterolysis (path A), resulting in the oxoMn(V) species **1** and nitrite ion, or a homolytic O−O bond scission (path B) that generates the oxoMn(IV) intermediate **2** and $NO_2^{\bullet}$.[22]

(21) Cleavage inhibition was a function of the amount of NO_2^-. Conditions were identical to those of the cleavage reactions, except MnTMPyP (1 μM) and NO_2^- concentrations were varied between 100 μM and 100 mM.

The NO_2^- and $NO_2^{\bullet}$ species from path A and path B (Scheme 1) are not innocent, and we find that they direct the reactivity of the oxoMn intermediates toward the nitration of added phenols. Low levels of MnTMPyP efficiently catalyzed the nitration of hydroxyphenylacetic acid (HPA) by peroxynitrite to give HPA−NO_2.[23,24] A proton-catalyzed heterolysis (path C) affording Mn^{III}−OH (**3**) and NO_2^+ can effect nitrations, but this is unlikely in light of the observed stoichiometric oxidation of MnTMPyP by $ONOO^-$. Either oxidation of the incipient NO_2^- by an oxoMn(V) intermediate (**1**) or path B could generate oxoMn(IV) (**2**) and $NO_2^{\bullet}$. Nitration of the phenol at this stage is likely to result from the known phenoxy radical coupling to $NO_2^{\bullet}$.[25]

Support for this mechanism derives from the observed *nitration* of HPA by MnTMPyP−HSO_5^- in the presence of NO_2^-.[23] HSO_5^- reacts with MnTMPyP via O−O bond heterolysis to afford **1**.[26] Subsequent oxidation of nitrite by **1** would produce **2** and $NO_2^{\bullet}$, the same species generated by the MnTMPyP−$ONOO^-$ system responsible for nitrations. Accordingly, we suggest that the oxidative DNA cleavage and phenol nitration reactions proceed via the oxoMn(V) and oxoMn(IV) intermediates,[16a,b] respectively, generated by oxygen transfer from peroxynitrite.

It is clear that these metalloporphyrin complexes efficiently catalyze the decomposition of $ONOO^-$ and mediate the oxidative cleavage of plasmid DNA. An oxoMn(IV) intermediate (**2**) is observed. Protection from cleavage by nitrite ion and direction of the reactivity toward phenolic nitration can be understood to result from the interception of an oxoMn(V) species (**1**) by NO_2^-. These observations may have important consequences regarding the pathophysiology of $ONOO^-$. Adventitious metal ions known to be present under conditions of cell stress[11,27] would accelerate oxidations and nitrations of cellular components in regions of high $ONOO^-$ flux, thereby potentiating its toxicity. Alternatively, metal complexes designed to intercept reactive oxygen intermediates in cells could provide an avenue for pharmacological intervention.[28]

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(22) Reduction of the oxoMn species **1** and **2** by buffer can result in the generation of a MnTMPyP species (**4**) (Scheme 1).

(23) Nitration of HPA was initiated by the addition of $ONOO^-$ or HSO_5^- (0.5−1 mM) to a mixture of the metalloporphyrin (2.5−25 μM) and HPA (1 mM) in a 50 mM phosphate buffer (pH 7.4). The NO_2^- concentrations in the HSO_5^- system were varied between 1 and 100 mM with MnTMPyP (10 μM), resulting in HPA−NO_2 (8%−60% yields). In the $ONOO^-$ system, yields of HPA−NO_2 were between 30% and 60%, depending on the MnTMPyP concentration. Less than 8% was observed without catalyst. All yields were determined by HPLC and are reported with respect to the HPA. Other substrates tested were phenol and tyrosine, which gave similar results.

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