Mn(II)−Texaphyrin as a Catalyst for the Decomposition of Peroxynitrite

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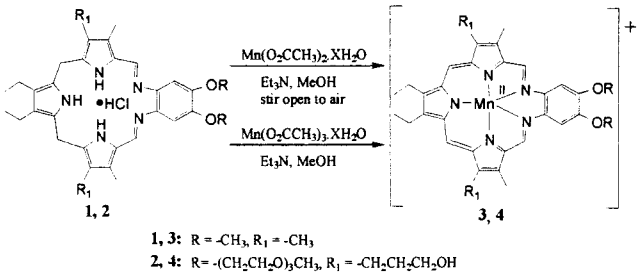
Manganese and iron porphyrins,[1−3] as well as other macrocyclic metal complexes,[4] have recently been reported to be highly active catalysts for peroxynitrite decomposition. Peroxynitrite anion, $ONOO^-$, formed in vivo by combination of nitric oxide and superoxide anion,[5] has been implicated as a cytotoxic agent in connection with numerous conditions and diseases including atherosclerosis,[6] ALS,[7] cancer,[8] and ischemia−reperfusion injury.[9] It is believed that peroxynitrite forms RNSs (reactive nitrogen species) during its decay into less reactive nitrate and nitrite anions, and it is these RNSs that react with biological targets.[10] The resulting damage includes oxidation of lipids and nitration of tyrosine residues and DNA.[11] Metalloproteins are particularly sensitive to peroxynitrite anion due to rapid oxidation of iron and manganese centers.[12] Therefore, finding synthetic metal complexes that can act catalytically and safely to decompose peroxynitrite without forming RNSs would constitute an important pharmacological advance. In this communication, we report the synthesis of the first structurally characterized Mn(II)−texaphyrin complex[13] (Mn-Tex) and its ability to catalyze the decomposition of peroxynitrite without causing concomitant phenol nitration in aqueous solution at pH 7.4.

Mn(II)−texaphyrin complexes were synthesized from the reduced texaphyrin precursors, **1** and **2**, using two different strategies as shown in Scheme 1. Briefly, the reduced form of the macrocycle was stirred in basic methanol solution with either

Scheme 1



Mn(II) acetate for several hours open to the air or Mn(III) acetate for approximately 1 h either under an inert atmosphere or open to the air. In both cases, the reduced Schiff base macrocycle was simultaneously oxidized and metalated to give the corresponding aromatic manganese texaphyrin complex, **3** or **4**.[14] Exchanging the counteranion from chloride to acetate enhanced the aqueous solubility of **4**, a species already equipped with solubilizing poly(ethylene glycol) substituents. This complex was used for all kinetic studies.

The manganese complexes **3** and **4** displayed UV−vis spectra characteristic of aromatic, metalated texaphyrins,[14] with a Soret-like band at 460 nm ($\log \epsilon = 4.96$, MeOH) and a *Q*-like band at 727 nm ($\log \epsilon = 4.51$, MeOH).

Several characterization methods were used to assign the oxidation state of the Mn(II) center in complexes **3** and **4**. Magnetic moment measurements, performed using the Evans method,[15] yielded a calculated spin-only magnetic moment of approximately 6.0 μ_B for **3** and **4**, typical of a high-spin Mn(II) metal center.[16] Additionally, the rich EPR spectrum of **4**, recorded at liquid N_2 temperature, was typical of Mn(II) complexes, displaying prominent peaks at 5.03, 2.65, and 1.83 g, as well as characteristic fine structure. Finally, a single-crystal X-ray diffraction analysis of **3** was performed.[17] The resulting structure, shown in Figure 1, reveals the presence of only one axially coordinated chloride counteranion, as would be expected of a Mn(II)−texaphyrin complex.

The structure of **3**, the first to be reported for a transition metal texaphyrin complex, differs from the others obtained previously using group 12 or Ln(III) cations.[14] The metal center is displaced from the mean intersection point of the five nitrogen atoms and is shifted toward the three pyrrolic nitrogens (Mn−N(pyrrole) = 2.226(3), 2.383(3), 2.233(3) Å; Mn−N(imine) = 2.520(3), 2.558(3) Å). The Mn(II) center is also found to reside slightly above the macrocyclic framework, 0.371(1) Å from the plane defined by the five N atoms.[17]

Reactions of **4** with $ONOO^-$ were studied in 0.1 M phosphate buffer at pH 7.4 using a rapid-mixing stopped flow diode array spectrophotometer, monitoring the reaction from 300 to 500 nm. This allowed both the decay of $ONOO^-$ (λ_{max} at 302 nm) and the dynamic behavior of the texaphyrin Soret-like band to be followed concurrently. As shown in Figure 2, after mixing **4** and $ONOO^-$, the absorbance maxima at 302 and at 460 nm were seen to decrease. In addition, a new broad peak emerged at 400 nm. We have assigned this latter spectral feature to the corresponding Mn(III)−texaphyrin intermediate, based in part on its reversion to

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(17) Crystal data for **3-Cl:** $(C_{34}H_{38}N_5O_2)MnCl{\cdot}CH_2Cl_2$, $M_r = 724.01$, monoclinic, $P2_1/c$, $a = 13.1921(3)$ Å, $b = 22.1216(7)$ Å, $c = 12.1975(4)$ Å, $\beta = 105.289(2)°$, $T = 123(2)$ K, $Z = 4$, $V = 3433.6(2)$ Å^3, 4650 reflections with $I > 2\sigma(I)$ used, $R = 0.0665$, and $R_w = 0.130$.

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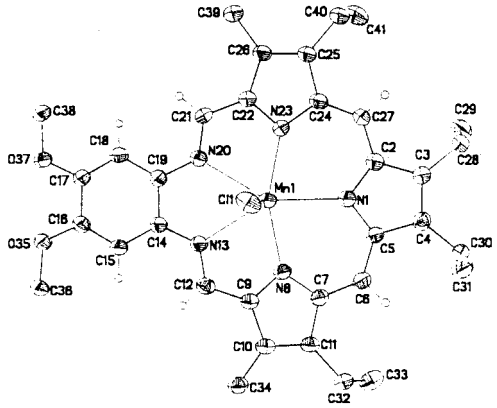


Figure 1. View of **3-Cl** showing the atom labeling scheme. Thermal ellipsoids are scaled to the 50% probability level. Hydrogen atoms shown are drawn to an arbitrary scale.

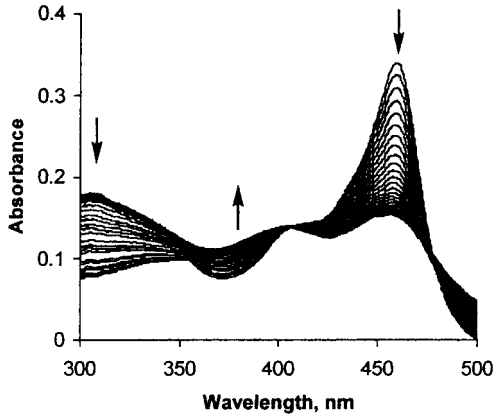


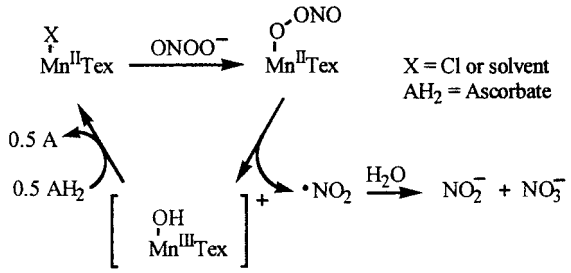
Figure 2. Reaction of 5 μM **4** with 60 μM $ONOO^-$. During the course of the reaction, as the peroxynitrile decomposes, the Mn(II) peak at 460 nm decreases, and a new peak at 400 nm, assigned to a Mn(III) complex, emerges. The presence of an isosbestic point at 410 nm is interpreted in terms of a fast conversion to this latter species without the formation of a long-lasting intermediate.

the starting complex in the presence of a reductant. When this intermediate was allowed to react with 0.5 equiv of ascorbate, nearly complete reversion to Mn(II)-Tex is observed. In this case, ascorbate provides two electron equivalents upon its oxidation to dehydroascorbate. To verify the oxidation state of the intermediate, we also obtained its EPR spectrum at 6 K. The spectrum lacked the signal typical of Mn(IV), further supporting the assignment of the Mn(III) state. The rate constant for Mn(II)−Mn(III) oxidation by $ONOO^-$ is estimated at 3×10^4 M^{-1} s^{-1}, as judged from the plot of pseudo-first order k_{obs}, obtained from decay at 460 nm, versus [**4**]. In the absence of strong reducing agents, such as ascorbate, compound **4** did not catalytically decompose $ONOO^-$ due to very slow reduction of the Mn(III) intermediate back to Mn(II) by residual nitrite, present as 10−20% impurity in peroxynitrite. In this manner, the behavior of Mn−texaphyrin parallels that of Mn porphyrins, studied previously.[3]

However, when reducing agents such as ascorbate are added to Mn−texaphyrin during decomposition of $ONOO^-$, the Mn−texaphyrin decomposed peroxynitrite catalytically.[18] In the presence of 50 μM ascorbate, the rate of $ONOO^-$ decay was found to increase by nearly a factor of 2. A plot of the pseudo-first-order rate constant (k_{obs}) versus ascorbate concentration revealed that low concentrations (25−100 μM) of the reductant accelerated the reaction by reducing the metal center. However, a large excess

(18) Ascorbate reacts with peroxynitrite a rate of 85 M^{-1} s^{-1}. At these concentrations of the reactants, the contribution to the overall decay rate is negligible.

Scheme 2



of ascorbate, relative to the catalyst, (e.g. 500 μM) was found to be detrimental, due to the ligation of ascorbate to the catalyst (K_d = 78 μM).

On the basis of our knowledge of the interaction of $ONOO^-$ with manganese and iron porphyrins, we propose the catalytic cycle illustrated in Scheme 2. Peroxynitrite anion reacts with the Mn(II) texaphyrin to form an adduct that then decomposes via homolysis of the O−O bond to give a Mn(III)−texaphyrin and nitrogen dioxide ($k_1 = 3 \times 10^4$ M^{-1} s^{-1}). Nitrogen dioxide is a reactive nitrogen compound involved in nitration of phenols. However, in aqueous solution it is rapidly hydrolyzed to form nitrate and nitrite anions at an overall rate constant of 5×10^7 M^{-1} s^{-1};[3] therefore, it is inefficient at nitration by itself. The Mn(III)−texaphyrin, produced concurrently, is reduced back to the starting Mn(II) complex by nitrite (in which case the cycle is not catalytic), a process which is accelerated by adding optimal amounts of ascorbate, completing the catalytic cycle.

A key aspect of the above mechanism is the suggestion that **4** acts to decompose $ONOO^-$ in such a way that benign daughter products are produced. Consequently, as outlined in greater detail elsewhere,[1] the presence of **4** could offer greater protection against $ONOO^-$ in a biological milieu than a simple analysis of k_{cat} would lead one to predict. As a preliminary test of this hypothesis, we examined the peroxynitrite-mediated nitration of fluorescein, a probe for nitration by $ONOO^-$,[19] in the presence of **4**.[20] The yield of nitrofluorescein, as measured by the change in absorbance at 490 nm, was reduced by nearly 2-fold (from 33 to 19%) in the presence of **4**. By contrast, Mn(III)TMPyP has been reported to enhance phenol nitration.[2d] Compared to the porphyrin complexes previously studied, **4** appears to be one of the more active nitration inhibitors.[3,21] Its effectiveness is on par with some of the more active Fe porphyrins, for example, FeTMPS, which are known to protect tyrosines from nitration by $ONOO^-$ in vivo.[2c] This leads us to suggest that **4** and related texaphyrin complexes could prove useful for the pharmacological inactivation of $ONOO^-$.[22]

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Supporting Information Available: UV−vis and EPR spectra, all X-ray data tables, kinetic data and plots (PDF). This material is available free of charge via Internet at http://pubs.acs.org.

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(22) (a) Because manganese porphyrins and other complexes have shown superoxide dismutase activity, the ability of Mn−Tex to decompose superoxide was tested. This was done using stopped flow rapid-mixing spectrophotometry according to the published procedure.[22b] Under the conditions employed, the second-order rate constant for superoxide decay was measured to be 1.0×10^5 M^{-1} s^{-1} independent of [Mn−Tex]. On the basis of this, we conclude that the upper limit of the catalytic rate constant, if any, for superoxide dismutation by Mn−Tex is = 10^6 M^{-1} s^{-1}. (b) Riley, D. P.; Rivers, W. J.; Weiss, R. H. *Anal. Biochem.* **1991**, *196*, 344−349.