Corroles

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Iron and Manganese Corroles Are Potent Catalysts for the Decomposition of Peroxynitrite**

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Peroxynitrite, which is formed in vivo by the reaction of NO and O_2^-, is more toxic than all other isolable reactive oxygen species because there are no specific enzymes for preventing its spontaneous decay to radical-decomposition products that damage cellular molecules such as DNA, peptides, proteins, sugars, and lipids.[1–4] Strong evidence points toward peroxy nitrite as being heavily involved in many biological malfunctions initiated by oxidative stress,[5] and the same holds for neurodegenerative disorders that (among many others) include Alzheimer's, Parkinson's, and Huntington's diseases.[6–10] The damage-inducing properties of peroxynitrite are known to derive from the homolytic bond cleavage of its protonated form (HOONO, dominant at physiological pH) to $^{\bullet}OH$ and $^{\bullet}NO_2$ [Eq. (1)].[3–5] These strong oxidizing and

$$HOONO \rightarrow {}^{\bullet}OH + {}^{\bullet}NO_2 \quad (1)$$

nitrating species cause irreversible damage and/or chemical modification to almost any biomolecule,[11] thus highlighting the urgent need to develop effective bioavailable compounds that will catalyze the transformation of peroxynitrite into

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benign products [Eqs. (2) and (3)].

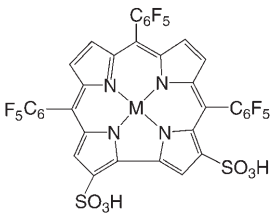
$$HOONO \rightarrow H^+ + NO_3^- \quad (2)$$

$$2\,HOONO \rightarrow 2\,H^+ + 2\,NO_2^- + O_2 \quad (3)$$

The seminal findings that initiated much research in this field were the stoichiometric and catalytic decomposition of peroxynitrite by water-soluble manganese(III) and iron(III) porphyrins, reported by Groves and Marla in 1995 and Stern et al. in 1996, respectively.[12] Metalloenzymes were also explored for the same purpose and some encouraging results were obtained in several in vivo investigations.[13] Another ongoing emphasis is on resolving the mechanism of action, mainly through determination of the rate constants of elementary steps relevant to the overall catalytic process.[14] It is now clear that there are two distinct pathways for detoxification of peroxynitrite: isomerization to nitrate [Eq. (2)] and disproportionation to nitrite and molecular oxygen [Eq. (3)].[15]

Many details regarding the mechanism of catalysis by metalloporphyrins are now resolved, with most indications pointing toward elementary steps that involve only one-electron redox processes (M^{III} and M^{IV}; M = Fe, Mn).[14–17] The (oxo)iron(IV) intermediates react further with the decomposition products of peroxynitrite and thus complete a catalytic cycle. Although (oxo)manganese(IV) porphyrins are formed very fast, by either homolytic O–O scission or by the reduction of (oxo)manganese(V) intermediates by NO_2^-,[12a] they do not react further with either peroxynitrite or any of its products and are hence limited to single-turnover reactions.[13] Catalytic decomposition of peroxynitrite by manganese(III) porphyrins is only achievable in the presence of stoichiometric amounts of sacrificial antioxidants.[17]

The recent disclosures of facile methods for the synthesis of corroles paved the way to research that revealed the great potential of the corresponding metal complexes as catalysts for quite a variety of reactions.[18–20] Of particular relevance to this study are the amphiphilic corrole **1** and its metal complexes (Scheme 1).[21] They associate very strongly to proteins,[22] and the noncovalently conjugated iron(III) and manganese(III) corroles (**1**-Fe and **1**-Mn, respectively) catalyze the peroxidase-like enantioselective oxidation of sulfides and the catalase-like decomposition of hydrogen peroxide to water and molecular oxygen.[23a] The last phenomenon triggered our interest in the reactions of metallocorroles



1: M = 3H
1-Fe: M = Fe^{III}
1-Mn: M = Mn^{III}

Scheme 1. Amphiphilic corrole **1** and its metal complexes.

with other reactive oxygen species, of which peroxynitrite was selected as the highest priority target for the reasons outlined above. Herein, we show that the catalytic rate for decomposition of peroxynitrite by **1**-Fe is significantly larger than that of analogous porphyrins and second only to the most-optimized positively charged iron porphyrin. The more important result is that **1**-Mn also displayed catalytic activity, contrary to all other manganese complexes. The results further illustrate that catalysis by **1**-Mn is a genuine disproportionation process [Eq. (3)], which proceeds through reactions that do not create any radical species.

The investigations started by examining the decay of peroxynitrite at pH 7.4 and 25 °C in the presence and absence of various amounts of catalyst. The results shown in Figure 1

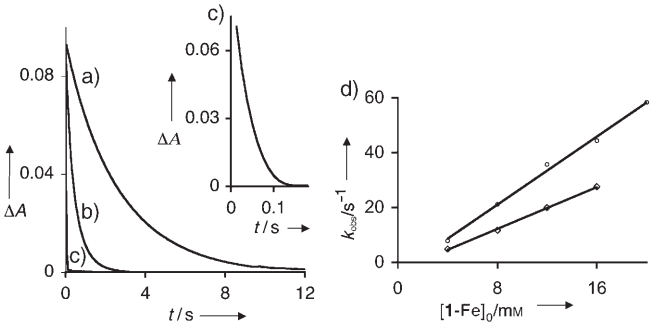


Figure 1. a–c) Decomposition of 385 μM peroxynitrite at pH 7.4 and 25 °C monitored at $\lambda = 302$ nm, the λ_{max} of peroxynitrite, without catalyst (a), or in the presence of 16 μM (4 mol%) **1**-Mn (b) or **1**-Fe (c; shown at two timescales). d) Observed decomposition rates of peroxynitrite (385 μM, pH 7.4) as a function of **1**-Fe concentration at 25 °C (lower trace) and 37 °C (upper trace).

(curves a–c) are representative of the first-order decays that were obtained in all cases. The results obtained without catalyst, that is, spontaneous decay of peroxynitrite with an apparent first-order rate constant of 0.38 s^{-1}, are practically identical to those of previous reports under comparable conditions.[12–17] In the presence of the catalysts the half-life of peroxynitrite was dramatically shortened from 1.8 to 0.31 and 0.025 s with **1**-Mn and **1**-Fe, respectively. Plots of rate constants at various catalyst concentrations revealed linear relationships (Figure 1, right) and the thus-elucidated k_{cat} values were 4.0×10^4 (**1**-Mn, 25 °C), 8.6×10^4 (**1**-Mn, 37 °C), 2.0×10^6 (**1**-Fe, 25 °C), and 3.1×10^6 $M^{-1}s^{-1}$ (**1**-Fe, 37 °C). The catalytic rate of **1**-Fe is two to ten times higher than values reported for the most-active iron porphyrins ($k_{cat} = 0.3$ to 1.6×10^6 $M^{-1}s^{-1}$ at 37 °C),[24] except for the one reported by Szabó et al. ($k_{cat} = 6.25 \times 10^6$ $M^{-1}s^{-1}$ at 25 °C).[13b] The catalytic performance of **1**-Mn (almost 1900 turnovers, see below) is most novel, when considering that all other manganese complexes (including complexes with porphyrins) reported to date do not catalyze the reaction without the aid of sacrificial agents.[13,17]

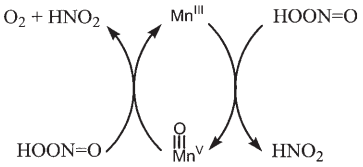
The amount of nitrite formed was monitored to distinguish between the possible modes for the decomposition of peroxynitrite [Eqs. (1)–(3)]. The yield was almost quantitative (95% NO_2^- relative to $^-OON{=}O$) with **1**-Mn as the

catalyst, whereas that obtained under catalysis by **1**-Fe (25% NO_2^-) was even less than the yield from the spontaneous decay of peroxynitrite (30% NO_2^-). These results clearly suggest that the main modes of catalytic action are different: isomerization [Eq. (2)] for **1**-Fe and disproportionation [Eq. (3)] for **1**-Mn. The latter conclusion gains further support from the very efficient disproportionation of hydrogen peroxide by **1**-Mn,[23a] considering that both reactions share the same essential requirements. The substrate (H_2O_2 or HOONO) must be able to act as a two-electron oxidant of manganese(III) to (oxo)manganese(V), as well as a two-electron reductant of the latter. These two elementary steps are outlined in Equations (4) and (5) for peroxynitrite, and

$$\text{HOONO} + \textbf{1}\text{-Mn}^{\text{III}} \rightarrow \text{H}^+ + \text{NO}_2^- + \textbf{1}\text{-Mn}^{\text{V}}(\text{O}) \quad (4)$$

$$\text{HOONO} + \textbf{1}\text{-Mn}^{\text{V}}(\text{O}) \rightarrow \text{H}^+ + \text{NO}_2^- + \text{O}_2 + \textbf{1}\text{-Mn}^{\text{III}} \quad (5)$$

the corresponding catalytic cycle [consistent with the stoichiometry of Eq. (3)] is drawn in Scheme 2.



Scheme 2. Proposed catalytic cycle for disproportionation of peroxynitrite catalyzed by manganese(III) corrole.

The above proposal was tested by simultaneously recording spectral changes in the UV (peroxynitrite decomposition) and visible parts of the electronic spectrum (possible time-dependent changes in the structure of the catalyst). At pH 7.4, the decay at 302 nm was not accompanied by any changes in the visible spectrum of **1**-Mn (Figure 2a). This rules out the accumulation of reaction intermediates with lifetimes that would allow detection at the 3-ms intervals that were used. Nevertheless, strong evidence for the involvement of the (oxo)manganese(V) intermediate **1**-Mn(O) (accessible by oxidation of **1**-Mn by iodosylbenzene)[23a] was obtained in separate experiments. The decay of independently prepared **1**-Mn(O) was found to be quite slow in aqueous solutions of pH 7.4 (Figure 2b), but addition of peroxynitrite at any time caused its immediate reduction to **1**-Mn. Secondly, **1**-Mn(O) was the steady-state intermediate when the reaction between peroxynitrite and **1**-Mn was carried out at high pH value (Figure 2c), conditions under which (oxo)manganese(V) complexes are much less reactive.[23b,c] These results add significant confidence to the proposed elementary steps described in Equations (4) and (5) and the catalytic cycle drawn in Scheme 2. Apparently, the reactions of peroxynitrite with both **1**-Mn [Eq. (4)] and the (oxo)manganese(V) intermediate [Eq. (5)] are very fast at physiologically relevant pH values. The results shown in Figure 2a and c also highlight the stability of the catalyst, which is neither bleached nor irreversibly modified, as is the case with iron enzymes.[25] The most critical test was performed by quantifying the

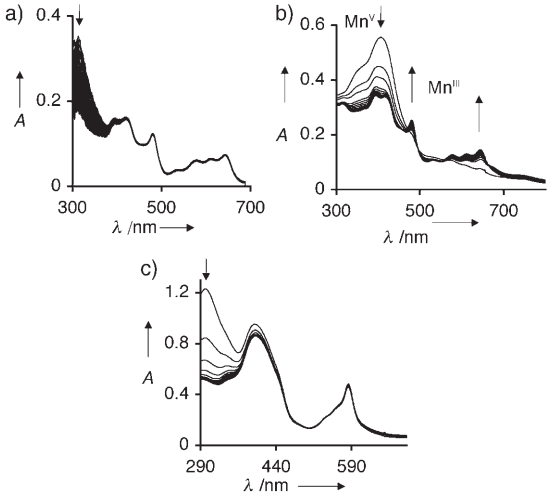


Figure 2. Time-resolved diode-array absorption spectra (25 °C) recorded at a) 3-ms intervals (0–0.3 s) during the reaction of **1**-Mn (40 μM) with peroxynitrite (1400 μM) at pH 7.4; b) at 20-s intervals (0–200 s) during the slow transformation of the (oxo)manganese(V) complex **1**-Mn(O) to **1**-Mn at pH 7.4; and c) at 10-s intervals (0–110 s) during the reaction of **1**-Mn (36 μM) with peroxynitrite (720 μM) at pH 12.7. Note: the spectrum of independently prepared **1**-Mn(O) is identical to that obtained by the reaction of **1**-Mn with peroxynitrite at the same pH value.

amount of molecular oxygen released in a relatively large-scale reaction,[26] which corresponded to a chemical yield of 98% O_2 and 1875 catalytic turnovers of **1**-Mn.

Examination of the **1**-Fe-catalyzed reaction by similar means revealed much more complicated phenomena. Decomposition of peroxynitrite (385 μM, 11 μM catalyst, 302 nm) displayed a first-order decay with a half lifetime of 38 ms (reaction is 94% complete within 0.15 s), while changes in the visible part of the spectrum were clearly inconsistent with one major species in a steady-state concentration. For example, there was an intensity increase at 432 nm in the first 2 ms that was followed by a nonexponential decay extending to 0.3 s. This result is reminiscent of the iron porphyrin system, where multiple pathways contribute to the overall catalytic cycle and its efficiency.[16] Accordingly, the complicated task of elucidating which particular elementary steps are responsible for the fast catalysis by iron corroles was postponed to future studies.

The complexes introduced in this work catalyze the decomposition of peroxynitrite very efficiently. The iron corrole is among the fastest-performing catalysts reported to date and approaches the speed of the most-active positively charged porphyrin.[13b] All results are consistent with disproportionation, by the mechanism shown in Scheme 2, as the dominant mechanism for catalysis by the manganese complex. The striking contrast between the results obtained with manganese(III) complexes of porphyrins and the corrole serves to emphasize the main advantage of the new catalyst. The less-reducing porphyrin-chelated manganese(III) complexes are oxidized by peroxynitrite to (oxo)manganese(IV) porphyrins, which are not reactive enough toward peroxynitrite and/or its decomposition products. However, two-

electron oxidation of manganese(III) corroles is a facile process,[27] and the (oxo)manganese(V) corroles obtained are of sufficient reactivity for oxidizing peroxynitrite and thus completing the catalytic cycle.

This difference is of fundamental importance as regards the possible utilization of such catalysts in medicinal applications. While catalytic systems that rely on one-electron redox processes may be very useful for synthetic purposes,[28] the radical species that are involved are clearly prohibitive in biochemical environments. Recent demonstrations of the low cytotoxicity of these corroles and their facile, noncovalent conjugation to targeting proteins[29] further highlight the great potential of these compounds as catalysts for the prevention of peroxynitrite-induced damage to biological molecules. We assume that these findings will have a profound impact on the possible use of metallocorroles in preventive therapeutic treatments. One focus in forthcoming research is on increasing the catalytic rate to reach that obtained by the combination of metalloporphyrins and sacrificial reducing agents.[17]

Experimental Section

1-Fe and **1**-Mn were prepared by reported procedures.[21–23] Peroxynitrite was prepared from the reaction of sodium nitrite with acidified hydrogen peroxide according to reported procedures, which included a treatment for destroying excess H_2O_2.[30] Nitrite was quantified by the Griese reagent.[16] Time-resolved visible spectra were recorded on an Applied Photophysics PiStar-180 spectrometer in the photodiode-array fast-scan mode. Reaction kinetics were collected in the photomultiplier mode on the PiStar-180 spectrometer, using a single mixing mode. One syringe contained peroxynitrite (770 μM) in NaOH (50 mM), and the other contained phosphate buffer with or without the catalysts. Equal volumes were injected into a stopped-flow cell, and the pH value of the reaction mixture was measured at the outlet. The configuration of the instrument permitted continuous flow of water from a thermostated bath over the flow path and firing syringes containing the reagents. Peroxynitrite decay was monitored at 302 nm. First-order decay rates (k_{obs}) were fitted by standard techniques with software provided by the instrument manufacturer.

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[26] A peroxynitrite solution (0.15 M, 2 mL) was added to a phosphate buffer solution of **1**-Mn (2.66×10^{-5} M, 6 mL, final pH of 7.4) at 25 °C and the amount of O_2(g) was quantified volumetrically as 3.6 mL. This result translates to 49% relative to peroxynitrite, which corresponds to a chemical yield of 98% with respect to Equation (3) and 1875 catalytic turnovers.
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