Electrostatic Contribution in the Catalysis of O$_2^-$ Dismutation by Superoxide Dismutase Mimics. Mn$^{III}$TE-2-PyP$^{5+}$ vs Mn$^{III}$Br$^T$-2-PyP$^+$

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**Abstract**

The Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin, Mn$^{III}$TE-2-PyP$^{5+}$, is a potent SOD mimic *in vitro*, and was beneficial in rodent models of oxidative stress pathologies. Its high activity has been ascribed to both the favorable redox potential of its metal center and to the electrostatic facilitation assured by the four positive charges encircling the metal center. Its comparison with the non-alkylated, singly-charged analogue Mn(III) *beta*-octabromo *meso*-tetrakis(2-pyridyl)porphyrin, Mn$^{III}$Br$_8$T-2-PyP$^+$, enabled us to evaluate the electrostatic contribution to the catalysis of O$_2^-$ dismutation. Both compounds exhibit nearly identical metal-centered redox potential for Mn$^{III}$/Mn$^{II}$ redox couple: +228 mV for Mn$^{III}$TE-2-PyP$^{5+}$ and +219 mV vs NHE for Mn$^{III}$Br$_8$T-2-PyP$^+$. The eight electron-withdrawing *beta* pyrrolic bromines contribute equally to the redox properties of the parent Mn$^{III}$T-2-PyP$^+$ as do four quaternized cationic *meso* ortho pyridyl nitrogens. However, the SOD-like activity of the highly charged Mn$^{III}$TE-2-PyP$^{5+}$ is 200-fold higher ($\log k_{cat} = 7.76$) than that of the singly-charged Mn$^{III}$Br$_8$T-2-PyP$^+$ ($\log k_{cat} = 5.63$). The kinetic salt effect showed that the catalytic rate constants of the Mn$^{III}$TE-2-PyP$^{5+}$ and of its methyl
analogue, Mn$^{III}$TM-2-PyP$^{5+}$, are exactly 5-fold more sensitive to ionic strength than is the $k_{\text{cat}}$ of Mn$^{III}$Br$\gamma$T-2-PyP$^+$, which parallels the charge ratio of these compounds. Interestingly, only a small effect of ionic strength on the rate constant was found in the case of penta-charged para (Mn$^{III}$TM-4-PyP$^{5+}$) and meta isomers (Mn$^{III}$TM-3-PyP$^{5+}$), indicating that the placement of the positive charges in the close proximity of the metal center (ortho position) is essential for the electrostatic facilitation of O$_2^-$ dismutation.

**Introduction**

The thermodynamic (1,2-8) and electrostatic effects (9,10-15) in the catalysis of O$_2^-$ dismutation by superoxide dismutases have been extensively studied. The redox potential of all superoxide dismutases was found to be similar, independently of the type of the metal in the active site. It is around midway (+360 mV vs NHE) (16) between the potential for the oxidation (-160 mV vs NHE) and for the reduction of O$_2^-$ (+890 mV vs NHE). Thus it allows equal driving force (hence $k_{\text{ox}} = k_{\text{red}} = \sim 2 \times 10^9$ M$^{-1}$ s$^{-1}$) for both half-reactions of the catalytic cycles (eqs [1] and [2]) (17-19). For *E. coli* FeSOD

\[
\text{Mn}^{III}\text{SOD} + \text{O}_2^- \rightleftharpoons \text{Mn}^{II}\text{SOD} + \text{O}_2 \quad k_{\text{ox}} \quad [1]
\]

\[
\text{Mn}^{II}\text{SOD} + \text{O}_2^- + 2\text{H}^+ \rightleftharpoons \text{Mn}^{III}\text{SOD} + \text{H}_2\text{O}_2 \quad k_{\text{red}} \quad [2]
\]
the \( E_{1/2} \) is +223 mV vs NHE at pH 7.4, and for MnSOD from the *B. stearothermophilus* and *E. coli* the \( E_{1/2} \) were +260 and +310 mV vs NHE at pH 7 (1,20,21). However, when manganese was replaced by iron in the active site of MnSOD the enzymatic activity was lost (17), which has been attributed to the decrease of the redox potential below that required for the oxidation of superoxide ion (3,17). Such metal ion specificity (2) has been recently explained by the higher affinity of Fe\(^{3+}\) than Mn\(^{3+}\) for hydroxide (6,8).

The crystal structures of different SODs reveal a highly conserved electrostatic “funnel” (12,13,15) that is believed to guide the negatively charged superoxide towards the active site of the enzyme. In the past there have been considerable efforts to evaluate the extent of electrostatic facilitation, but a major difficulty lies in the inability to specifically modify the positively charged residues without affecting the structural integrity of the active site (9).

The Mn(III) porphyrin, Mn\(^{III}\)TE-2-PyP\(^{5+}\) (AEOL-10113) has been shown (22-26) to possess high SOD-like activity *in vitro* with \( \log k_{\text{cat}} = 7.76 \). The compound has further proven effective in protection of SOD-deficient *E. coli* (25) and in stroke (27,28), spinal cord injury (29), diabetes (30,31), sickle cell disease (32), and radiation/cancer (33,34) rodent models of oxidative stress injuries. Much like SOD (1,9,20,21) its high catalytic potency has been ascribed both to the favorable redox properties of the metal center and to the effect of the positively charged *ortho* N-ethylpyridyl nitrogens that provide electrostatic facilitation for the approach of the negatively charged superoxide (22).
The Mn$^{III}$TE-2-PyP$^{5+}$ (Scheme I) exists as a mixture of rotational isomers (35). Expectations that the four positive charges in $\alpha\alpha\alpha\alpha$ isomer will guide the superoxide anion towards the metal center in a cooperative fashion making it the most powerful SOD mimics among the isomers, proved to be groundless; all four isomers were found to be of equal catalytic potency.

Recently, the synthesis and characterization of the $\beta$-brominated non-$N$-alkylated analogue of Mn$^{III}$TE-2-PyP$^+$ has been reported (36). The electron-withdrawing effect of the $\beta$-pyrrolic bromines on the redox properties of the metal center of the porphyrins (50-70 mV/bromine) has been previously established (37-43). The effect of eight $\beta$-pyrrolic bromines was expected to be similar in magnitude to the effect of the four quaternized pyridyl nitrogens on the redox properties of the starting unsubstituted Mn$^{III}$TE-2-PyP$^+$ porphyrin molecule.

Herein, we show that the redox properties of the brominated non-$N$-alkylated and the $N$-alkylated Mn(III) ortho pyridylporphyrins are indeed nearly identical, allowing us to evaluate the electrostatic contribution in the catalysis of $O_2^-$ dismutation. Similar studies would be hard to conduct on the enzymes themselves. Thus our findings give us the unique opportunity to understand the relative importance of thermodynamics and kinetics in the $O_2^-$ dismutation by the superoxide dismutases (17-19).

**Experimental**

**Materials**
**General.** Xanthine and ferricytochrome c were from Sigma, and NaCl, KOH, KH$_2$PO$_4$, methanol and EDTA from Mallinckrodt. Xanthine oxidase was prepared by R. Wiley and was supplied by K. V. Rajagopalan (44). Catalase was from Boehringer, ultrapure argon from National Welders Supply Co., and tris buffer (ultrapure) was from ICN Biomedicals, Inc.

**Mn(III) porphyrins.** The H$_2$T-2-PyP$^+$ and Mn$^{III}$TM-3(4)-PyP$^5+$ were obtained from MidCentury Chemicals (Chicago, IL). Mn$^{III}$TE(M)-2-PyP$^5+$ (25,26) and Mn$^{III}$Br$_8$T-2-PyP$^+$ (36) were prepared as previously described. The molar absorptivities of the Soret bands of MnTM-2-PyP$^5+$ ($\log \epsilon_{453.4} = 5.11$), MnTM-3-PyP$^5+$ ($\log \epsilon_{459.8} = 5.14$), MnTM-4-PyP$^5+$ ($\log \epsilon_{462.2} = 5.11$), MnTE-2-PyP$^5+$ ($\log \epsilon_{454} = 5.14$) (25,26) all in water and of Mn$^{III}$Br$_8$T-2-PyP$^+$ ($\log \epsilon_{482} = 4.66$) in acetonitrile were used for quantitation. Due to the low water-solubility, a 2mM stock solution of Mn$^{III}$Br$_8$T-2-PyP$^+$ in methanol was used throughout this study.

**Methods**

**Electrochemistry.** Measurements were performed on a CH Instruments Model 600 Voltammetric Analyzer. A three-electrode system was utilized with a glassy carbon (3 mm) or gold (2mm) button working electrode (Bioanalytical Systems), a Ag/AgCl reference and a Pt wire as auxiliary electrode. Due to the low water-solubility of the
Mn$^{III}$Br$_3$T-2-PyP$^+$, electrochemical studies of both compounds were performed in 9/1 (v/v) methanol/aqueous solutions as previously reported (45a). The 9/1 (v/v) methanol/aqueous solutions contained 0.05 M tris, pH 7.8, 0.1 M NaCl, and 0.3 mM metalloporphyrin. Tris buffer was used instead of phosphate buffer because the latter precipitates in methanol. The potentials were standardized against potassium ferrocyanide/ferricyanide (46) and Mn$^{III}$TE-2-PyP$^{5+}$. The redox potential of the Mn$^{III}$/Mn$^{IV}$ redox couple which was previously found to be proton-dependent (47) was determined at pH 12.3. The scan rates were 0.01-10 V/s. $E_{1/2}$ for Mn$^{II}$/Mn$^{III}$ and Mn$^{III}$/Mn$^{IV}$ redox couples obtained in 9/1 (v/v) methanol/aqueous solutions were extrapolated to aqueous medium values as previously described (45a).

**Catalysis of O$_2$·⁻ dismutation.** We have previously shown that the convenient cytochrome c assay gives the same catalytic rate constants as does pulse radiolysis in the case of Mn$^{III}$TE-2-PyP$^{5+}$, {Mn$^{III}$BVDME}$_2$, {Mn$^{III}$BV$^2$⁻}$_2$ and Mn$^{II}$Cl$_2$ (45a) and it was therefore utilized in this study. The xanthine/xanthine oxidase reaction was the source of O$_2$·⁻ and ferricytochrome c was used as the indicating scavenger for O$_2$·⁻ (48). The reduction of cytochrome c was followed at 550 nm. Assays were conducted at (25±1) °C, in 0.05 M phosphate buffer, pH 7.8, 0.1 mM
EDTA, 10 µM cytochrome c, 40 µM xanthine, ± 15 µg/mL of catalase. Aqueous stock solutions of MnIII TE(M)-2-PyP5+ and MnTM-3(4)-PyP5+ and the methanolic stock solution of MnIII BrgT-2-PyP+ were diluted into the assay mixture. Rate constants for the reaction of metalloporphyrins with O2•− were based upon competition with 10 µM cytochrome c as described elsewhere (45a). The kcytc = 2.6 x 105 M−1 s−1 obtained under the same experimental conditions (pH 7.8, 21 ºC, 0.05 M phosphate buffer, 0.1 mM EDTA) (45b) was used to calculate kcat. The O2•− was produced at the rate of 1.2 µM per minute. Any possible interference through inhibition of the xanthine/xanthine oxidase reaction by the test compounds was examined by following the rate of urate accumulation at 295 nm in the absence of cytochrome c. No reoxidation of ferrocytochrome c by metalloporphyrins was observed. No effect of catalase was detected implying sufficient stability of the compounds towards H2O2. We have previously determined the rate constants for the degradation of MnIII TE-2-PyP5+ and of MnIII TM-2-PyP5+ by H2O2 to be 1.3 M−1 s−1 and for MnIII TM-3-PyP5+ and MnIII TM-4-PyP5+ 4.9 and 4.6 M−1 s−1, respectively. In this work we found that the MnIII BrgT-2-PyP+ proved to be at least two orders of magnitude more stable.

Kinetic salt effect. The dependence of the catalytic rate constant for the O2•− dismutation upon ionic strength was determined in 0.05 M phosphate buffer, pH 7.8 with
NaCl ranging from 0 to 0.4 M.

Results

Electrochemistry. The Mn\textsuperscript{II}/Mn\textsuperscript{III} redox couple. Reversible cyclic voltammograms of the Mn\textsuperscript{II}/Mn\textsuperscript{III} redox were obtained for both compounds, Mn\textsuperscript{III}TE-2-PyP\textsuperscript{5+} and Mn\textsuperscript{III}Br\textsubscript{8}T-2-PyP\textsuperscript{+}, at scan rates of 0.1 V/s (Figure 1). Thus it was possible to determine the half-wave potentials, $E_{1/2}$, given in Table 1. The two compounds have almost identical Mn\textsuperscript{II}/Mn\textsuperscript{III} metal centered $E_{1/2}$ values at pH 7.8, as predicted from the number and the nature of the electron-withdrawing substituents on the meso positions of the porphyrin ring (37-43). Moreover, their voltammetric behavior in terms of electrochemical reversibility (peak-to-peak potential separation, $\Delta E_{pp}$, Figure 2A, and current response to a change in scan-rate, Figure 2C) as well as the chemical reversibility (ratio between the reduction and oxidation peak currents, Figure 2B) is strikingly similar which leaves us to believe that the difference in the reactivity towards superoxide is indeed due to the difference in the overall positive charge (electrostatic attraction of superoxide anion) and not due to a difference in the rates of electron transfer (electronic and structural differences).

The Mn\textsuperscript{III}/Mn\textsuperscript{IV} redox couple. Redox properties of the Mn sites were further explored by studying Mn\textsuperscript{III}/Mn\textsuperscript{IV} redox couple. Because the hydroxo-Mn\textsuperscript{III} and oxo-Mn\textsuperscript{IV} species are involved (47), this redox process is accessible only in basic solution.
and is proton-dependent. At pH 12.3, reversible cyclic voltammograms were obtained in the case of both compounds, Mn^{III}TE-2-PyP^{5+} and Mn^{III}Br_{2}T-2-PyP^{+}, with essentially equal Mn^{III}/Mn^{IV} redox couple potentials of +381 mV and +372 mV vs NHE, respectively (Figure 1B, Table 1). The Mn^{III}/Mn^{IV} and Mn^{II}/Mn^{III} redox processes are independent as demonstrated on Figure 1B (dashed traces) whereas reversible voltammetric waves were obtained even when the cycling was done only in a narrow potential range around the E_{1/2} of the corresponding redox couple.

Because at pH 12.3 a deprotonation of the axially ligated water on Mn(III) porphyrins occurs, the Mn^{II}/Mn^{III} redox potential shifts negatively (47). Therefore, we ascribe the 145 mV difference in the shift between the two redox couples (Figure 1B, Table 1) to a difference in the pK_{a,ax}s of their axially ligated water. We have previously found that E_{1/2} reflects the electron density of the porphyrin ring and the metal center in such a way that there is a linear relationship between the pK_{a} of the pyrrolic nitrogen protons of the porphyrin ligand and the metal-centered E_{1/2} for a series of differently substituted Mn porphyrins (25). We have further found that the axial ligation also is influenced by the electron density of the metal center; in the case of ortho, meta and para MnTM-2-PyP^{5+} more positive E_{1/2} correlates with lower pK_{a,ax} (47). However, in the case of a series of Mn(III) ortho N-alkylpyridylporphyrins (alkyl = methyl through octyl) we saw that hydrophobic effects may reverse the trend (49). With more hydrophobic members of the series, despite a more positive E_{1/2}, the creation of charge is hindered
resulting in higher pK_a s of the pyrrolic nitrogens of the parent ligands (49). In the present work at pH 12.3, the hydrophobic Mn^{III}BrgT-2-PyP^+ with presumably higher pK_a,ax (thus resisting deprotonation), exhibits a lower shift of the Mn^{II}/Mn^{III} couple than highly hydrophilic Mn^{III}TE-2-PyP^5+. In the case of Mn^{III}/Mn^{IV} couple which is also proton-dependent (47), there was practically no difference in E_{1/2} between the two compounds which is in line with findings reported by us (23,45-47) and others (50-56) that Mn^{III}/Mn^{IV} redox potential is fairly insensitive to the porphyrin structure.

**Catalysis of O_2^- dismutation.** The catalytic rate constants were calculated from the linear plots of v_0/v_{i-1} vs concentration obtained from the spectrophotometric cytochrome c assay measurements, as described elsewhere (45a). The k_{cat} values determined in 0.05 M phosphate buffer, pH 7.8 are given in Table 1. The Mn^{III}BrgT-2-PyP^+ is ~200-fold less efficient a catalyst than Mn^{III}TE-2-PyP^5+.

**Kinetic salt effect.** The effect of the ionic strength (\(\mu\)) on the catalytic rate constant was assessed using eq [3] which is based on Debye-Huckel relation (57) for the effect of the ionic strength of the solution on the activity coefficient of an ion.

\[
\log k = \log k_{\text{ref}} + 2 A z_A z_B (\mu^{1/2}/1 + \mu^{1/2})
\]
The $k$ is the rate constant at any given ionic strength, while $k_{\text{ref}}$ is the rate constant at $\mu = 0$. The $A$ is a collection of physical constants with a value of 0.509 and $z_A$ and $z_B$ are the charges of the reacting species. The equation predicts a linear plot of $\log k$ vs $(\mu^{1/2}/1 + \mu^{1/2})$. Eq [3] assumes a coefficient of 1.0 ($\beta a_i$) for $\mu^{1/2}$ in the denominator, i.e., the distance of the closest approach, $a_i$ to be 3 Å and $\beta$ is a physical constant, $0.33 \times 10^{-10}$ m$^{-1}$. It is doubtful whether great significance can be attributed to the $a_i$, thus to the product $z_A z_B$ (35), especially so in the light of the bulkiness, high charge and solvation shell of the metalloporphyrins. Accounting for the mono- and diprotonated phosphates as the major species at pH 7.8 ($pK_a = 7.2$), and the concentration of the NaCl, the ionic strength was calculated using equation [4], where $m_i$ is the molality and $z_i$ the charge of the given ion.

$$\mu = \frac{1}{2} \Sigma m_i z_i^2$$  \hspace{1cm} [4]

Linear plots of $\log k_{\text{cat}}$ vs $\mu^{1/2}(1 + \mu^{1/2})$ (eq [3]) are presented in Figure 3. The slopes of the plots are -6.96 (Mn$^{III}$TE-2-PyP$^{5+}$), -6.93 (Mn$^{III}$TM-2-PyP$^{5+}$), -2.57 (Mn$^{III}$TM-3-PyP$^{5+}$), -2.60 (Mn$^{III}$TM-4-PyP$^{5+}$) and -1.41 (Mn$^{III}$Br$_8$T-2-PyP$^+$$)$. The intercepts present $k_{\text{ref}}$ and are 9.71 (Mn$^{III}$TM-2-PyP$^{5+}$), 9.68 (Mn$^{III}$TE-2-
PyP$^{5+}$), 7.25 (Mn$^{III}$TM-3-PyP$^{5+}$ and Mn$^{III}$TM-4-PyP$^{5+}$) and 6.01 (Mn$^{III}$Br$_8$T-2-PyP$^+$. As expected (49), when the reactants are ions of opposite charges, the higher the ionic strength of the solution the lower the rate constants. Methyl and ethyl ortho isomers, Mn$^{III}$E(M)-2-PyP$^{5+}$ behave equally with respect to kinetic salt effect. The ratio of slopes for Mn$^{III}$TE(M)-2-PyP$^{5+}$ and Mn$^{III}$Br$_8$T-2-PyP$^+$ was found to be 4.9, which equals the ratio of their charges. The kinetic salt effect of meta and para isomers, Mn$^{III}$TM-3-PyP$^{5+}$ and Mn$^{III}$TM-4-PyP$^{5+}$, was also assessed. Only a small effect of the positive charges was observed when they were placed peripherally with respect to metal center in para and meta isomers, Mn$^{III}$TM-3(4)-PyP$^{5+}$ (Figure 3).

**Discussion**

Log $k_{cat}$ vs $E_{1/2}$. In order to design a potent, low-molecular weight SOD mimic we aimed at approaching the $E_{1/2}$ of the enzyme and affording electrostatic facilitation. We established a relationship between the log $k_{cat}$ and the $E_{1/2}$ for the Mn$^{II}$/Mn$^{III}$ redox couple for a series of Mn(III) porphyrins. An increase in $E_{1/2}$ of 120 mV caused a 10-fold increase in $k_{cat}$ (25) which is in agreement with Marcus equation for an outer-sphere electron transfer (58,59). At potentials that are negative with respect to the midway potential, a Mn$^{+3}$ oxidation state is stabilized, and the reduction of...
metalloporphyrin becomes rate-limiting. The preliminary data indicate that, similar to
the SODs, when $E_{1/2}$ approaches the midway potential for $O_2^-$ reduction and oxidation
of $\sim +360 \text{ mV vs NHE}$, the rate constants for the reduction (eq [1]) and oxidation of Mn
porphyrins (eq [2]) by $O_2^-$ become similar (60). Thus the reduction of $\text{Mn}^{III}\text{TM-4-PyP}^5+$ at $E_{1/2} = +62 \text{ mV vs NHE}$, is 1000-fold slower than its oxidation (60), while the
reduction of $\text{Mn}^{III}\text{TE-2-PyP}^5+$, at $E_{1/2} = +228 \text{ mV vs NHE}$, is only $\leq 4$-fold slower
than its oxidation. Thus in a preliminary pulse radiolysis study the rate constant for the
reduction of $\text{Mn}^{III}\text{TE-2-PyP}^5+$ by $O_2^-$ was found to be $2.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ while it
was $8.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for its oxidation (61). As the potential increases further, the Mn
$+2$ oxidation state becomes stabilized and the oxidation of metalloporphyrin becomes
rate-limiting (5,20,62). Mn(II) porphyrins can efficiently catalyze dismutation of $O_2^-$. The $\text{Mn}^{II}\text{Br}^8\text{TM-4-PyP}^4+$ and $\text{Mn}^{II}\text{Cl}_5\text{TE-2-PyP}^4+$ do so with $\log k_{cat} = 8.34$ (20)
and 8.41 (62), respectively. However both compounds suffer from insufficient
metal/ligand stability to be used $\textit{in vivo}$ as SOD mimics (37,62).

Based on the $\log k_{cat}$ vs $E_{1/2}$ relationship, the $\text{Mn}^{III}\text{TE-2-PyP}^5+$ was chosen as
the most promising compound for $\textit{in vivo}$ testing. It dismutes $O_2^-$ with high catalytic
rate constant of $5.8 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at $E_{1/2}$ of $+228 \text{ mV vs NHE}$, and it affords
electrostatic facilitation in the catalysis while retaining metal/ligand stability.
**Deviation from the plot log k\text{cat} vs E_{1/2}.** Although dismutation appears to be an outer-sphere electron transfer, electrostatic, steric and solvation effects, which are not accounted for by the Marcus equation, do play a role. When one or more of these effects predominate over E_{1/2}, deviation from Marcus plot occurs. The highest degree of deviation was observed with the series of Mn(III) ortho N-alkylpyridyl porphyrins and with the singly charged Mn^{III}Br_{8}T-2-PyP^{+}. In the case of the former porphyrins, an interplay of steric and solvation effects results in a “V” shape dependence of log k\text{cat} upon E_{1/2} (49). Thus, as the alkyl chains lengthen from methyl to n-octyl accompanied by an increase in hydrophobicity, the E_{1/2} steadily increases. Yet, from methyl to n-butyl the k\text{cat} decreases, while it increases from n-butyl to n-octyl. In the case of singly-charged Mn^{III}Br_{8}T-2-PyP^{+}, as discussed below, the deviation from the Marcus plot originates from the lack of electrostatic facilitation.

**Electrostatics vs redox potential in the catalysis of \text{O}_2^{-} dismutation by SOD mimics.** Like Mn^{III}TE-2-PyP^{5+}, the singly charged Mn^{III}Br_{8}T-2-PyP^{+} is a derivative of the same parent compound, Mn^{III}T-2-PyP^{+}. When eight electron-withdrawing bromines are placed on the β-pyrrolic positions of Mn^{III}T-2-PyP^{+} they cause a positive shift in E_{1/2} of the Mn^{II}/Mn^{III} redox couple of 499 mV (Table 1 and Figure 1). Our finding is in agreement with available literature on the effect of β-bromination on the
E$_{1/2}$ of metalloporphyrins (37-43). An almost identical increase in E$_{1/2}$ (508 mV) was achieved by placing four quaternized ortho pyridyls in the meso positions of the Mn$^{III}$T-2-PyP$^+$. Thus the E$_{1/2}$ of Mn$^{III}$TE-2-PyP$^5+$ and Mn$^{III}$Br$_8$T-2-PyP$^+$ are +228 mV and +219 mV vs NHE, respectively (Table 1 and Figure 1). Even though the E$_{1/2}$ values for the Mn$^{II}$/Mn$^{III}$ redox couple responsible for O$_2^{-}$ dismutation are essentially identical, Mn$^{III}$TE-2-PyP$^5+$ was found to be a 200-fold more efficient catalyst of O$_2^{-}$ dismutation. The corresponding log $k_{cat}$ are 7.76 and 5.63 for Mn$^{III}$TE-2-PyP$^5+$ and Mn$^{III}$Br$_8$T-2-PyP$^+$, respectively (Table 1). Thus we conclude that the effect of such a magnitude originates entirely from electrostatic facilitation of the O$_2^{-}$ dismutation.

As an additional support for the importance of the electrostatics in the O$_2^{-}$ dismutation, the kinetic salt effect was assessed in order to see whether a 5-fold charge difference causes a 5-fold higher susceptibility of the catalytic rate constant of Mn$^{III}$TE-2-PyP$^5+$ to the ionic strength when compared to the $k_{cat}$ of the singly-charged Mn$^{III}$Br$_8$T-2-PyP$^+$. The linear plots of log $k_{cat}$ vs $\mu^{1/2}(1 + \mu^{1/2})$ (eq [1]), obtained for both compounds (Figure 3), show that the ratio of slopes (4.9) equals the ratio of charges, which clearly establishes the impact that electrostatics has on the SOD-like catalysis. The same effects of the ionic strength on $k_{cat}$ were observed in the case of ethyl and
methyl analogues, Mn\textsubscript{III}TE(M)-2-PyP\textsuperscript{5+} (Figure 3). The \textit{meta} and \textit{para} isomers, Mn\textsubscript{III}TM-3(4)-PyP\textsuperscript{5+}, were also studied in order to determine the importance of the location of the positive charges. Very little salt effect was observed when the charges are further away from the metal center; thus the \textit{meta} and \textit{para} isomers behaved much as did the Mn\textsubscript{III}BrgT-2-PyP\textsuperscript{+} (Figure 3). Therefore, close proximity of the positive charges is essential for O$_2^-$ guidance. These data strongly point to the metal center as the site of electron transfer, and suggest that the dismutation might not be truly outer-sphere in that there might be some bonding interactions during the encounter of the reactants.

**Abbreviations**

SOD, superoxide dismutase; NHE, normal hydrogen electrode; \textit{meso} refers to the substituents at the 5,10,15, and 20 (\textit{meso} carbon) position of the porphyrin core; \textit{β} refers to the substituents at \textit{β}-pyrrolic carbons; Mn\textsubscript{III}T-2-PyP\textsuperscript{+}, Mn(III) 5,10,15,20-tetrakis(2-pyridyl)porphyrin; Mn\textsubscript{III}TE-2-PyP\textsuperscript{5+} (Mn-2E\textsuperscript{+}) (AEOL-10113), manganese(III) 5,10,15,20-tetrakis(N-ethylpyridinium-2-yl)porphyrin; Mn\textsubscript{III}TM-2(3,4)-PyP\textsuperscript{5+} (Mn-2(3,4)M), manganese(III) 5,10,15,20-tetrakis(N-methylpyridinium-2(3,4)-yl)porphyrin, where 2 (AEOL-10112), 3, and 4 refer to \textit{ortho}, \textit{meta} and \textit{para} isomers, respectively; Mn\textsubscript{III}BrgT-2-PyP\textsuperscript{+} (Mn-Brg\textsuperscript{+}), Mn(III) 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(2-pyridyl)porphyrin; Mn\textsubscript{II}BrgTM-4-PyP\textsuperscript{4+}, Mn(II)
2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin; Mn$^{II}$Cl$_5$TE-2-PyP$^{4+}$, Mn(II) β-pentachloro-5,10,15,20-tetrakis(N-ethylpyridinium-2-yl)porphyrin; {Mn$^{III}$BV$^{2-}$}$_2$, Mn(III) biliverdin IX; {Mn$^{III}$BVDME}$_2$, Mn(III) biliverdin IX dimethyl ester.

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Schemes

Scheme I. Structures of the Mn\textsuperscript{III}TE(M)-2-PyP\textsuperscript{5+}, Mn\textsuperscript{III}TM-3-PyP\textsuperscript{5+}, Mn\textsuperscript{III}TM-4-PyP\textsuperscript{5+} and Mn\textsuperscript{III}Br\textgreek{g}T-2-PyP\textsuperscript{+}.

Figures

Figure 1. (a) Cyclic voltammograms of Mn\textsuperscript{III}TE-2-PyP\textsuperscript{5+} (Mn-2E\textsuperscript{5+}) and Mn\textsuperscript{III}Br\textgreek{g}T-2-PyP\textsuperscript{+} (Mn-Br\textgreek{g}\textsuperscript{+}) obtained at pH 7.8 in 9/1 (v/v) methanol/aqueous solution, 0.05 M tris buffer, 0.1 M NaCl, scan rate 0.1 V/s. (b) Cyclic voltammograms of Mn\textsuperscript{III}TE-2-PyP\textsuperscript{5+} (Mn-2E\textsuperscript{5+}) and Mn\textsuperscript{III}Br\textgreek{g}T-2-PyP\textsuperscript{+} (Mn-Br\textgreek{g}\textsuperscript{+}) obtained at pH 12.3 in 9/1 (v/v) methanol/aqueous solution, 0.05 M tris buffer, 0.1 M NaCl, scan rate 0.1 V/s. First cycle solid line, subsequent cycles dashed lines.

Figure 2. Electrochemistry of Mn\textsuperscript{III}TE-2-PyP\textsuperscript{5+} (Mn-2E\textsuperscript{5+}) and Mn\textsuperscript{III}Br\textgreek{g}T-2-PyP\textsuperscript{+} (Mn-Br\textgreek{g}\textsuperscript{+}) at pH 7.8 in 9/1 (v/v) methanol/aqueous solution, 0.05 M tris buffer, 0.1 M NaCl. (A) Peak-to-peak potential separation as a function of log (scan rate). (B) Ratio of the reduction and oxidation peak currents as a function of log (scan rate). (C) The reduction and oxidation peak currents as a function of the square root of the scan rate.
Figure 3. Log $k_{\text{cat}}$ vs $(\mu^{1/2}(1 + \mu^{1/2})$ for Mn$^{III}$TM-2-PyP$^{5+}$, Mn$^{III}$TM-3-PyP$^{5+}$, Mn$^{III}$TM-4-PyP$^{5+}$, Mn$^{III}$TE-2-PyP$^{5+}$ and Mn$^{III}$Br$_8$T-2-PyP$^+$ obtained in 0.05 M phosphate buffer, pH 7.8, 0-0.4 M NaCl. Slopes are given in parenthesis.

The intercepts present $k_{\text{ref}}$ and are 9.71 (Mn$^{III}$TM-2-PyP$^{5+}$), 9.68 (Mn$^{III}$TE-2-PyP$^{5+}$), 7.25 (Mn$^{III}$TM-3-PyP$^{5+}$ and Mn$^{III}$TM-4-PyP$^{5+}$) and 6.01 (Mn$^{III}$Br$_8$T-2-PyP$^+$).

Tables

Table 1. The $E_{1/2}$ for the Mn(II)/Mn(III) and Mn(III)/Mn(IV) Redox Couples and $k_{\text{cat}}$ for the $O_2^-$ Dismutation by Mn$^{III}$TE-2-PyP$^{5+}$ and Mn$^{III}$Br$_8$T-2-PyP$^+$.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_{1/2}$ (Mn(II)/Mn(III))$^a$</th>
<th>$E_{1/2}$ (Mn(III)/Mn(IV))$^b$</th>
<th>log $k_{\text{cat}}$$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mV vs NHE (vs Ag/AgCl)</td>
<td>mV vs NHE (vs Ag/AgCl)</td>
<td></td>
</tr>
<tr>
<td>Mn$^{III}$TE-2-PyP$^{5+}$</td>
<td>+228 (+132)</td>
<td>+381 (+285)</td>
<td>7.76</td>
</tr>
<tr>
<td>Mn$^{III}$Br$_8$T-2-PyP$^+$</td>
<td>+219 (+123)</td>
<td>+372 (+276)</td>
<td>5.63</td>
</tr>
</tbody>
</table>

$^a$ $E_{1/2}$ (±3 mV) were obtained in 9/1 (v/v) methanol/aqueous solution, 0.05 M tris buffer, pH 7.8, 0.1 M NaCl and extrapolated in aqueous solution as previously described (33). $^b$ $E_{1/2}$ (±3 mV) were obtained in 9/1 (v/v) methanol/NaOH aqueous solution, 0.1 M NaCl, pH 12.3 and extrapolated in aqueous solution as previously described (33). $^c$k$_{\text{cat}}$ (±5%) were determined at
(25 ± 1) °C by cytochrome c assay in 0.05 M phosphate buffer, pH 7.8
B

\[
\text{Mn-}2E^{5+} \\
\text{Mn-Br}_8^+ 
\]

\[\frac{i_{\text{red}}}{i_{\text{ox}}} \]

\[\log(\text{scan rate, V/s})\]
Electrostatic Contribution in the Catalysis of $\text{O}_2^{-}$ Dismutation by Superoxide Dismutase Mimics: $\text{Mn}^{III}\text{TE-2-PyP}^{5+}$ vs $\text{Mn}^{III}\text{Br8T-2-PyP}^{+}$
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