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THE INTERACTIONS OF SUPEROXIDE ION($O_2^{\cdot}$) WITH METALLO-PORPHYRINS [($C1_8$TPP)M, M = FE,MN,CO,ZN]; MODELS FOR BIOLOGICAL SYSTEMS AND SUPEROXIDE DISMUTASES

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In dimethylformamide superoxide ion forms a 1:1 adduct with tetrakis (2,6-dichlorophenyl) porphinato-iron, (Cl_8TPP)$FeOO^-$, as well as with its manganese analogue, (Cl_8TPP)$MnOO^-$. On the basis of their electrochemical, spectroscopic, and magnetic properties these adducts have a metal-oxygen covalent bond (PorM—OO^-), oxygen-centered redox chemistry, and reactivities that are similar to the hydroperoxide ion (HOO^-). Addition of ^-OH to a solution of PorFe and O_2 results in the formation of PorFe(OH)(OO^-), which can be electrochemically oxidized to PorFeOH plus O_2 (−0.2 V vs SCE). Addition of protons to the PorM-OO^- adducts promotes their rapid decomposition to PorM, HOOH, and O_2. This chemistry provides insight to the reactions of biological superoxide and superoxide dismutases.

KEY WORDS: Superoxide ion, metallo-porphyrins, iron-SOD, manganese-SOD.

Although there remains considerable question as to the amount (and mechanism of generation) of free superoxide ion ($O_2^{\cdot}$) in the cytosol of biological systems, the species has significant lifetime in aqueous media at pH 7 (~ 1 ms for 1 mM $O_2^{\cdot}$).[1] In the absence of catalysts or superoxide dismutase this lifetime is determined by the kinetics for the proton-induced disproportionation

$$O_2^{-\cdot} + HA \longrightarrow HOO\cdot + A^- \quad pK_{HOO\cdot}, 4.9 \qquad (1)$$
$$\xrightarrow[HA]{O_2^{-\cdot}} O_2 + HOOH + A^- \quad k, 10^8 M^{-1}s^{-1}$$

Even in the presence of unbuffered water $O_2^{-\cdot}$ is thermodynamically unstable

$$2\,O_2^{-\cdot} + H_2O \longrightarrow O_2 + HOO^- + HO^- \quad K, 10^8\,atm^{-1} \qquad (2)$$

Lewis acids other than protons [$Zn(ClO_4)_2$, $Fe(ClO_4)_2$, $Mn(ClO_4)_2$, and $Co(ClO_4)_2$] also facilitate the disproportionation of $O_2^{-\cdot}$ at faster rates and without the formation of the HOO· radical as an intermediate[1]

$$O_2^{\cdot} + Zn(ClO_4)_2 \longrightarrow Zn^+\text{-}OO\cdot + 2\,ClO_4^- \qquad (3)$$
$$\xrightarrow{O_2^{-\cdot}} O_2 + 1/n \left[Zn\text{-}OO \right]_n$$
$$\xrightarrow{2\,HA} HOOH + ZnA_2$$

Because the superoxide dismutase proteins contain transition metals (Fe, Mn, or Cu/Zn) there is a general belief that they facilitate the disproportionation of $O_2^{\cdot}$ via a metal-centered electron-transfer mechanism, e.g.,[2-4]

$$Fe^{III}L_m + O_2^{-\cdot} \xrightarrow{HA} L_nFe^{II} + O_2 + HL + A^- \qquad (4a)$$

$$L_nFe^{II} + O_2^{-\cdot} \xrightarrow[HL]{HA} Fe^{III}L_m + HOOH + A^- \qquad (4b)$$

However, several arguments have been presented that favor a radical-radical coupling between $O_2^{\overline{\cdot}}$ and an unpaired valence electron of the transistion metal center of SOD (Fe,Mn,Cu/Zn),[5,6]

$$Mn^{II}L_n + O_2^{-\cdot} \xrightarrow{HA} L_nMn\text{-}OOH + A^- \qquad (5a)$$

$$L_n\,Mn\text{-}OOH + O_2^{-\cdot} \xrightarrow{HA} Mn^{II}\,L_n + O_2 + HOOH + A^- \qquad (5b)$$

and thereby preclude the formation of the peroxy radical intermediates (HOO· and $M^+ - OO\cdot$) of the Lewis-acid mechanism and the oxidation-state change of the metal center for the electron-transfer mechanism. The resolution of these mechanistic alternatives requires that the SOD-$O_2^{\overline{\cdot}}$ intermediates be observed and characterized. The present study makes use of the tetrakis-meso-(2,6-dichlorophenyl)-porphyrinato-iron, -manganese, -cobalt, and -zinc complexes as models for the metal centers of SOD proteins. Because of steric barriers binuclear disproportionation paths are precluded

$$PorM(O_2^{-\cdot}) + PorM(O_2^{-\cdot}) \longrightarrow N\,R \qquad (6)$$

which makes it possible to stabilize and characterize the $O_2^{-\cdot}$ adducts of these metalloporphyrins.

Experimental Section

Equipment

Cyclic voltammetry was accomplished with a Bioanalytical Systems Model CV—27 and a Houston Instruments Model 200XY recorder. The electrochemical measurements were made with a microcell assembly (10—mL capacity) that was adapted to use a glassy-carbon working electrode (area, 0.09 cm^2), a platinum-wire auxiliary electrode (contained in a glass tube with a medium-porosity glass frit and filled with a concentrated solution of supporting electrolyte), and a Ag/AgCl reference electrode (filled with aqueous tetramethylammonium chloride solution and adjusted to 0.00 V vs SCE)[7] with a solution junction via a Pyrex glass tube closed with a cracked-glass bead (soft glass) that was contained in a luggin capillary.

Chemicals and Reagents

Dimethylformamide (DMF) and acetonitrile (MeCN), "distilled-in-glass" grade from Burdick and Jackson, were used without further purification. Tetraethylammonium perchlorate (TEAP) was vacuum-dried for 24 h prior to use. Tetrabutylammonium hydroxide [$(Bu_4N)OH$] was obtained form Aldrich as a 1 M solution in methanol, and its concentration was determined by acid-base titration. All other solvents and chemicals were the highest purity commercially available and were used as received.

Tetramethylammonium superoxide [$(Me_4N)O_2$] was prepared by combination of KO_2 and $(Me_4N)OH\cdot H_2O$ and subsequent extraction in liquid ammonia.[8,9] 5,10,15,20-Tetrakis(2,6-dichlorophenyl)porphine (Cl_8TPPH_2) was synthesized from

2,4,6-collidine[10,11] and was used to prepare $(Cl_8TPP)MnCl$,[12] $(Cl_8TPP)FeCl$,[11,12] $(Cl_8TPP)Co$,[12] and $(Cl_8TPP)Zn$.[10,13] The perchlorate salts, $(Cl_8TPP)Mn(ClO_4)$ and $(Cl_8TPP)Fe(ClO_4)$, were prepared by metathesis of the respective chloride salts with one equivalent of anhydrous $AgClO_4$ in hot toluene.[14]

RESULTS

The electrochemistry of O_2, and of O_2 in combination with $(Cl_8TPP)Zn$, $(Cl_8TPP)Fe$, $(Cl_8TPP)Mn$, and $(Cl_8TPP)Co$ is illustrated in Figure 1. The initial oxidation at 0.00 V

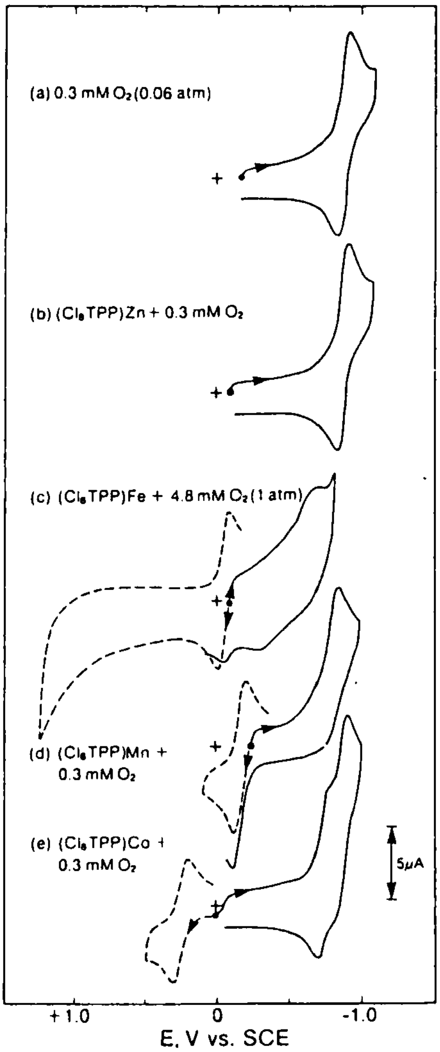


FIGURE 1 Cyclic voltammograms in DMF (0.1 M TEAP) of (a) 0.3 mM O_2 (0.06 atm); (b) 0.5 mM $(Cl_8TPP)Zn$ plus 0.3 mM O_2; (c) 0.5 mM $(Cl_8TPP)Fe$ plus 4.8 mM O_2 (1 atm); (d) 0.5 mM $(Cl_8TPP)Mn$ plus 0.3 mM O_2; and (e) 0.5 mM $(Cl_8TPP)Co$ plus 0.3 mM O_2 [scan rate 0.1 V s^{-1}; GCE].

for [(Cl_8TPP)Fe + O_2(1 atm)] is slightly more positive than that for (Cl_8TPP)Fe in the absence of O_2 ($E_{p,a}$, -0.02 V). Within twenty minutes after preparation this system hydrolyzes to give about 10% (Cl_8TPP)FeOH. In contrast, a solution of (Cl_8TPP)Fe and 1,2-dimethylimidazole in the presence of O_2 (1 atm) is stable for at least five hours.

The electrochemical oxidation of $^-$OH at a glassy-carbon electrode in DMF occurs at + 0.65 V vs SCE. In the presence of (Cl_8TPP)Zn, a transition-metal complex with a filled d^{10} subshell, the oxidation of $^-$OH occurs at essentially the same potential ($E^{\circ\prime}$, + 0.66 V). The formal reduction potential for the $O_2/O_2^{-\cdot}$ couple in DMF is

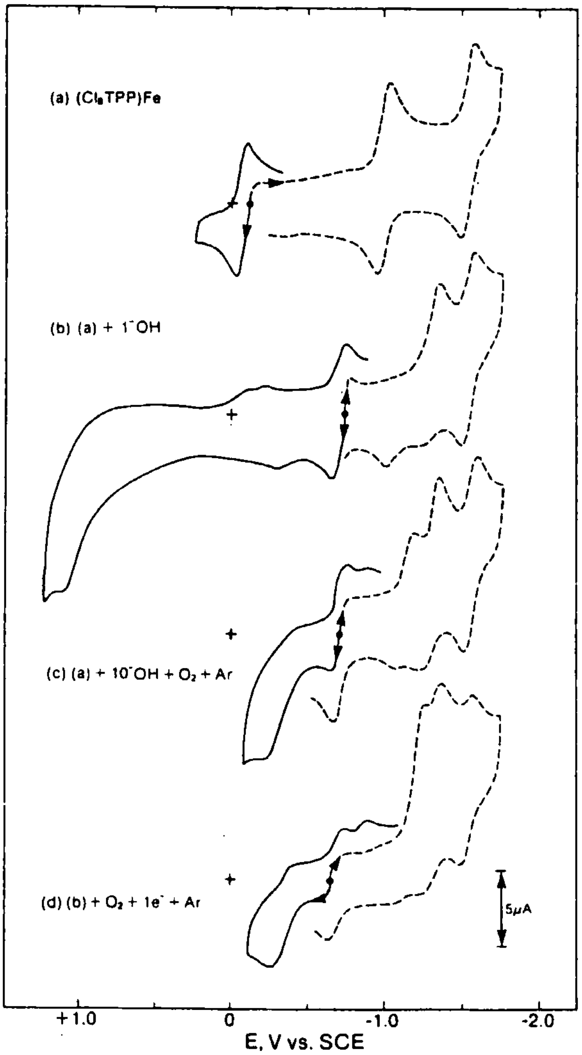
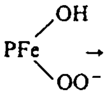


FIGURE 2 Cyclic voltammograms in DMF (0.1 M TEAP) of (a) 0.5 mM (Cl_8TPP)Fe; (b) 0.5 mM (Cl_8TPP)Fe plus 0.5 mM (Bu_4N)OH; (c) the product from the combination of 0.5 mM (Cl_8TPP)Fe, 0.5 mM (Bu_4N)OH, and O_2 (1 atm, *ca* 2 min.), followed by deaeration with Ar; and (d) deaerated one-electron reduction product of 0.5 mM (Cl_8TPP)Fe, one equivalent of (Bu_4N)OH, and O_2 (1 atm) [scan rate 0.1 V s^{-1}; glassy-carbon electrode (GCE)].

−0.8 V vs SCE; in the presence of $(Cl_8TPP)Zn$ with one equivalent of ^-OH, oxidation of $O_2^{-\cdot}$ occurs at −0.86 V.

The cyclic voltammograms of $(Cl_8TPP)Fe$ and its 1:1 combination with ^-OH are shown in Figures 2a and 2b, respectively. The combination of 10 equivalents of ^-OH with $(Cl_8TPP)Fe$ exhibits a cyclic voltammogram that is a composite of that for $[(Cl_8TPP)Fe + 1\ ^-OH]$ and that due to excess ^-OH (irreversible oxidations at 0.00 V and + 0.65 V). Bubbling O_2 (1 atm) through a solution of $[(Cl_8TPP)Fe + 10\ ^-OH]$, followed by deaeration with Ar, gives a product solution that exhibits the cyclic voltammogram shown in Figure 2c. Reversal of a positive scan after the oxidation

TABLE I
Redox reactions and thermodynamics for oxygen species and their adducts with $(Cl_8TPP)Fe$, $(Cl_8TPP)Mn$, and $(Cl_8TPP)Co$ in DMF[a]

	$E^{\circ\prime}$, V vs NHE[b]	$-\Delta G_{BF}$, kcal/mol[c]
$^-OH \rightarrow \cdot OH + e^-$	+0.92	
$2\ ^-OH \rightarrow O^{-\cdot} + H_2O + e^-$	+0.89	
$O^{-\cdot} \rightarrow \cdot O\cdot + e^-$	+0.67	
$O_2 + e^- \rightarrow O_2^{-\cdot}$	−0.64	
$O_2^{-\cdot} + H_2O + e^- \rightarrow H\text{-}OO^- + {}^-OH$	−1.40	72
$PFe + {}^-OH \rightarrow PFe\text{–}OH + e^-$ $(d^6sp);S = 4/2\ (d^6sp^2);S = 5/2$	−0.46	32
$PFe\text{–}OH + {}^-OH \rightarrow [(HO^-)PFe\text{–}OH]^-$ $(d^6sp^2);S = 3/2$	-	-
$PMn + {}^-OH \rightarrow PMn\text{–}OH + e^-$ $(d^6sp);S = 5/2\ \ (d^6sp);S = 4/2$	−0.19	26
$PCo + {}^-OH \rightarrow PCo\text{–}OH + e^-$ $(d^7sp);S = 1/2\ \ (d^7sp);S = 0$	−0.19	26
$PFe\text{–}OH + {}^-OH \rightarrow PFe{=}O + H_2O + e^-$ $(d^6sp);S = 2/2$	+0.24	-
$PMn\text{–}OH + {}^-OH \rightarrow PMn{=}O + H_2O + e^-$ $(d^6sp);S = 3/2$	< −0.19	
$PMn{=}O + 4\ {}^-OH \rightarrow$ $PMn{=}O + O_2 + 2\,H_2O + 4\,e^-$	+0.33	-
$PCo\text{–}OH + {}^-OH \rightarrow PCo{=}O + H_2O + e^-$ $(d^7sp);S = 1/2$	+0.67	-
$PFe\text{–}OO^- \rightarrow PFe + O_2 + e^-$ $(d^6sp^2);S = 5/2$	−0.12	12
 $\rightarrow PFe\text{–}OH + O_2 + e^-$	−0.09	13
PFe OH $OO^- \rightarrow PFe\text{–}OH + O_2 + e^-$ $(d^6sp);S = 2/2$	−0.09	13
$PMn\text{–}OO^- \rightarrow PMn + O_2 + e^-$ $(d^6sp);S = 4/2$	+0.11	17
$PCo\text{–}OO^- \rightarrow PCo + O_2 + e^-$ $(d^7sp);S = 0$	−0.31	8
$PFe(O_2) + e^- \rightarrow PFe\text{–}OO^-$ $(d^6sp);S = 0$	−0.33	-

[a] Proposed valence-electron hybridizations for the metal centers in parenthesis.

[b] $E_{NHE} = E_{SCE} + 0.24\ V$.

[c] $-\Delta G_{B.F.} = [E^{\circ\prime}_{X\cdot/X^-} - E^{\circ\prime}_{M\text{-}X/M,X^-}]$ 23.1 kcal. The $H\text{–}OO^-$ bond energy (ΔH_{DBE}) has a value of 80.1 kcal on the basis of electrochemical data (H_2O, pH 14); $O_2^{-}\cdot + H_2O + e^- \rightarrow H\text{–}OO^- + {}^-OH$ (E°, +0.20 V vs NHE), $HOH + e^- \rightarrow H\cdot + {}^-OH$ (E°, −2.93 V vs NHE), and $\Delta H_{DBE} = \Delta G_{BF} + T\Delta S_{DBE} = \Delta E^\circ$ (23.1 kcal/eV) + 7.8 kcal = 80.1 kcal.

peak at -0.31 V yields a new reduction peak at -0.88 V that is not observed for an initial negative scan. When one-to-three equivalents of ^{-}OH are combined with $(Cl_8TPP)Fe$ the addition of O_2 results in the formation of a product that has indentical electrochemistry to that for the 1:1 combination of $(Cl_8TPP)Fe(ClO_4)$ and OH.

Figure 2d illustrates the cyclic voltammogram for the solution that results from the combination of $(Cl_8TPP)Fe$, one equivalent of ^{-}OH, O_2 (1 atm) and one equivalent of electrons (controlled—potential coulometry at -0.96 V), and subsequent deaeration with Ar. Reversal of a positive scan after the -0.36-V oxidation peak results in a reduction at -0.90 V that is not observed for an initial negative scan. A product solution with identical electrochemical properties is obtained when two equivalents of tetramethylammonium superoxide $[(Me_4N)O_2]$ are combined [either with or without ^{-}OH present because dissolution of $(Me_4N)O_2$ yields $O_2^{-\cdot}$ and ^{-}OH][9] with $(Cl_8TPP)Fe(ClO_4)$ in DMF or MeCN. The results for similar experiments with $(Cl_8TPP)Mn$ and $(Cl_8TPP)Co$ are summarized in Table I.

DISCUSSION AND CONCLUSIONS

On the basis of the electrochemical, spectroscopic, and maganetic susceptibility results,[15] a self-consistent set of oxidation-reduction reactions for various oxygen species and their adducts with $(Cl_8TPP)Zn$, $(Cl_8TPP)Fe$, $(Cl_8TPP)Mn$, and $(Cl_8TPP)Co$ are proposed and the redox thermodynamics for the proposed metalloporphyrin-oxygen products are summarized in Table I. The dominant reaction of $O_2^{-\cdot}$ in nonacidic matrices is water-induced disproportionation ($2\,O_2^{-\cdot} + H_2O \rightarrow O_2 + HOO^- + HO^-$)[1]. Thus, any solvent system to which $O_2^{-\cdot}$ is added will produce substantial amounts of HO^-. Analysis of electrochemical results must include consideration of its redox chemistry first.

$^{-}$OH Adducts

The oxidation of ^{-}OH in the presence of $(Cl_8TPP)Zn$ occurs at a slightly more positive potenital ($+0.66$ V vs SCE) than that for free ^{-}OH ($+0.65$ V vs SCE). This is consistent with the metal center delocalizing the electron density for ^{-}OH to make removal of an electron more difficult. When ^{-}OH adducts of metalloporphyrins that contain partially filled d subshells are oxidized the potentials are less positive than that for free ^{-}OH oxidation. This facilitated oxidation of ^{-}OH is due to the stabilization of the oxidized product (·OH) via formation of a d-p covalent bond between the unpaired p electron of ·OH and an unpaired d electron of the metal center. This interpretation is consistent with previous reports that have demonstrated *d-p* covalent bond formation in the oxidation of ^{-}OH adducts of transition-metals[16] and metal-dithiolate complexes.[17]

Further support for this conclusion is provided by the magnetic data for the combination of $(Cl_8TPP)Fe(ClO_4)$ and ^{-}OH.[15] With the addition of one equivalent of ^{-}OH the $(Cl_8TPP)Fe(ClO_4)$ complex is reduced to $(Cl_8TPP)Fe$, which couples with the resulting ·OH to form $(Cl_8TPP)Fe$-OH. Reduction of PFe^+ by ^{-}OH has been demonstrated previously,[18,19] but the formation of a PFe-OH covalent bond and its electronic character have only recently been discussed.[15]

Recent theoretical[20–24] and experimental[16,17,24,25] reports have provided compelling arguments in support of covalent metal-ligand bonds in transistion-metal complexes.

Thus, metalloporphyrins (PZn, PMn, PFe, and PCo) are more reasonably formulated with uncharged metal-centers [Zn($d^{10}sp$), Mn(d^5sp), Fe(d^6sp), and Co(d^7sp)] bonded via two metal-nitrogen covalent bonds with uncharged porphyrin [analogous to porphine (PH_2)].[26,27] There is general acceptance that porphine has two hydrogen atoms bound via covalent bonds to two pyrrole nitrogens. The magnetic moment for (Cl_8TPP)Fe(OH) (5.15 B.M.) as well as other spectroscopic evidence[18] are consistent with d^5sp^2 hybridizaiton for the iron center of (Cl_8TPP)Fe–OH. As such two of the sp^2 electrons form two metal-pyrrole nitrogen covalent bonds and the remaining electron forms a metal-hydroxyl covalent bond (PFe-OH, bonding that is similar to that for H-OH and R-OH).

In the presence of excess $^-$OH an adduct is formed, [(HO^-)(Cl_8TPP)Fe—OH]$^-$, with the ligand field of $^-$OH inducing an in-plane octahedral geometry and an intermediate spin state (S = 3/2) (Table I). The electrochemical data (Figures 1 and 2 and Table I) and the magnetic data are consistent with the conclusion that oxidation of $^-$OH in the presence of (Cl_8TPP)Fe, (Cl_8TPP)Mn, and (Cl_8TPP)Co yields ·OH, which couples with an unpaired d electron and thereby reduces the magnetic moment of the metalloporphyrin by about one B.M.[15] (and the spin state by 1/2; see Table I). Thus, the redox and magnetic data support the conclusion that electron-transfer oxidation of the $^-$OH adducts for these metalloporphyrins is ligand-centered and facilitated by *d-p* covalent bond formation between the metal center and ·OH.

$O_2^{-\cdot}$ Adducts; PZn

The oxidation of $O_2^{-\cdot}$ in the presence of (Cl_8TPP)Zn occurs at a slightly more positive potential than that for free $O_2^{-\cdot}$. Because (Cl_8TPP)Zn (with $d^{10}sp$ valence-electron hybridization) does not offer any means to stabilize an $O_2^{-\cdot}$-adduct, $^-$OH is favored because of its greater basicity (larger change density on oxygen; the negative charge of $O_2^{-\cdot}$ is delocalized over both oxygens). Hence, there is no change in the UV-visible spectrum of (Cl_8TPP)Zn(OH) when $O_2^{\ \cdot}$ is introduced.

PFe

The sensitivity of PFe$(O_2)^-$ to degradation by trace levels of water ($2\,O_2^{-\cdot} + HOH \rightarrow HOO^- + O_2 + {}^-OH$) has been demonstrated.[28-30] In the present study the acidity of the medium[8,9] has been attenuated by the presence of excess $^-$OH, which enhances the stability of (Cl_8TPP)Fe$(O_2)^-$. The absence of reactions by this $O_2^{-\cdot}$ adduct with the solvent medium is confirmed by the essentially identical electrochemical and spectroscopic results obtained in DMF and MeCN; the latter has been successfully employed in the magnetic and spectroscopic characterization of (OEP)Fe$(O_2)^-$.[11] The UV-visble spectrum[15] of (Cl_8TPP)Fe$(O_2)^-$ is essentially the same as that for (TPP)Fe$(O_2)^-$ (TPP = tetraphenylporphyrin),[29,31] and its magnetic moment is similar to that for other PFe$(O_2)^-$ adducts.[28,29]

Oxidation of (Cl_8TPP)Fe$(O_2^{-\cdot})^-$ ($E^{\circ\prime}$, —0.36 V) occurs at a more positive potential than that for free $O_2^{-\cdot}$ ($E^{\circ\prime}$, – 0.88 V) [or for $O_2^{-\cdot}$ in the presence of (Cl_8TPP)Zn)]. This stabilization to electron removal is the result of covalent bond formation between an unpaired *p* electron of $O_2^{-\cdot}$ and an unpaired d electron of (Cl_8TPP)Fe to give (Cl_8TPP)Fe—OO^-, which is analogous to the stabilization of ·OH via coupling with (Cl_8TPP)Fe to give (Cl_8TPP)Fe-OH (both have iron centers with d^5sp^2 valence-electron hybridization and S = 5/2 spin states). The extent of stabilization, estimated by the shift in the $O_2^{-\cdot}$ oxidation potential (see Table I), is 12 kcal ($-\Delta G_{BF}$). The generation of O_2 upon oxidation of $(Cl_8TPP)Fe{-}OO^-$ is supported by its characteristic reduction at -0.88 V (Figure 2d). The irreversible reduction at -1.21 V is consistent with the dissociation of the oxygen species from $(Cl_8TTP)Fe$ to give ^-OOH, which reacts with Me_2SO to give Me_2SO_2 and ^-OH,[32] and with $(CH_3)CN$ to give $CH_3C(OH)(O^-)NH_2$.[9,33,34] Because (a) ^-OH is more difficult to oxidize than $O_2^{-\cdot}$ (Table I) and (b) oxidation of the ^-OH adducts of metalloporphyrins is ligand-centered, the oxidation of $(Cl_8TPP)Fe\text{-}OO^-$ must be ligand-centered (the liberation of O_2 upon a one-electron oxidation is consistent with this conclusion). The electron affinities for $O_2^{-\cdot}$ and $(Cl_8TPP)Fe^+$, which are directly proportional to their one-electron electron-transfer reduction potentials $[(Cl_8TPP)Fe^+$ ($E^{\circ\prime}_{DMF}$, $+0.18$ V vs NHE) $>$ O_2 (-0.64 V) $>$ $(Cl_8TPP)Fe$ (-0.74 V) $>$ $O_2^{-\cdot}$ (<-1.40 V)], preclude the formation of $[(Cl_8TPP)Fe^+(O_2^{2-})]^-$. Hence, there is no significant electron-transfer between $O_2^{-\cdot}$ and $(Cl_8TPP)Fe$ when they are combined.

The magnetic moment for $(Cl_8TPP)Fe(O_2)^-$ (5.57 B.M.)[15] and the absence of any propensity for outer-sphere electron-transfer between $(Cl_8TPP)Fe$ and $O_2^{-\cdot}$ (Table I), as well as other magnetic and spectroscopic evidence,[28,29] are consistent with d^5sp^2 hybridization for the iron center of $(Cl_8TPP)Fe(O_2)^-$. As such, the bonding is analogous to that for $(Cl_8TPP)Fe\text{-}OH$ with a metal-superoxide covalent bond ($PFe\text{-}OO^-$, bonding that is similar to that for $H\text{-}OO^-$ and $Bu\text{-}OO^-$).

The -0.18-V difference in the formal reduction potentials for the $(Cl_8TPP)Fe\text{-}OH/(Cl_8TPP)Fe(^-OH)^-$ couple ($E^{\circ\prime}$, -0.70 V) and $O_2/O_2^{-\cdot}$ couple ($E^{\circ\prime}$, -0.88 V), indicates that stabilization of $O_2^{-\cdot}$ via covalent-bond formation with $(Cl_8TPP)Fe\text{-}OH$ (32 kcal or more) will favor reduction of O_2 by $(Cl_8TPP)Fe(^-OH)^-$. Because the apparent $(Cl_8TPP)Fe\text{-}OO^-$ bond energy is 12 kcal ($-\Delta G_{BF}$, Table I), the combination of $(Cl_8TPP)Fe$ (d^6sp, $S = 4/2$), O_2, and ^-OH should result in the exothermic formation of $(Cl_8TPP)FeOHOO^-$ with Fe-OH and $Fe\text{-}OO^-$ covalent bonds. The observed magnetic moment (2.91 B.M., $S = 2/2$)[15] for the product solution from this combination, as well as the electrochemistry and UV-visible spectroscopy, are consistent with this formulation. Electrochemical oxidation at 0.0 V of the product solution yields O_2 and $(Cl_8TPP)Fe\text{-}OH$, which provides further support for the spontaneous formation of this species.

$$(Cl_8TPP)Fe + O_2 + {}^-OH \rightarrow (Cl_8TPP)FeOHOO^- \tag{7}$$

This reaction represents a unique and perhaps the first example of actual electron transfer between an iron porphyrin and dioxygen, and offers a means for oxygen transport and storage.

Because $(Cl_8TPP)Fe\text{-}OO^-$ and $(Cl_8TPP)FeOHOO^-$ are strong nucleophilic bases, excess ^-OH is required to stabilize them from reaction with trace levels of water in the solvent. In the absence of ^-OH reductive electrolysis of $[(Cl_8TPP)Fe + O_2]$ yields $(Cl_8TPP)Fe{-}OH$ as the major product, which results from a water-induced decomposition pathway (analogous to that for $O_2^{-\cdot}$, eq.(2))

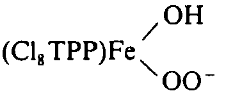
$$\begin{tikzcd}[column sep=small] (Cl_8TPP)Fe + O_2 + e^- \arrow[r] & (Cl_8TPP)Fe\text{-}OO^- \arrow[r, "H_2O"] & (Cl_8TPP)Fe\text{-}OOH + {}^-OH \arrow[d, "H_2O"] \\ & & (Cl_8TPP)Fe\text{-}OH + HOOH \end{tikzcd} \tag{8}$$

PMn and PCo

Electrochemical oxidation of $(Cl_8TPP)Mn(O_2)^-$ ($E^{\circ\prime}$, —0.13 V) liberates O_2, and the shift to a more positive potential relative to that for free $O_2^{-\cdot}$ is analogous to the behavior of $(Cl_8TPP)FeOO^-$. Thus, covalent bond formation between an unpaired d electron of PMn (d^5sp) provides stabilization of about 17 kcal (Table I).

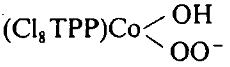
The observed magnetic moment for $(Cl_8TPP)MnOO^-$ (4.98 B.M.)[15] is the same as that for $(TPP)Mn(o_2),^-$[28] which has been formulated as $(TPP)Mn(O_2^{-\cdot})^-$.[28,35] A recent X-ray crystallographic structure determination[30] for $(TTP)Mn(O_2)^-$ has an O-O bond distance that is interpreted to be indicative of a O_2^{2-} dianion. However, earlier arguments for a reinterpretation of the vibrational data for $PFe(O_2)^-$ also apply to O-O bond distances.[15] Thus, the O—O bond distances should be similar for H-OO-H, (Na^{+-} OO ^{-+}Na), H—OO^-, and (TPP)Mn-OO^- (all with a bond-order of 1).

The generation of stable solutions of $(Cl_8TPP)MnOO^-$ by reduction of $[(Cl_8TPP)Mn + O_2]$ does not require the presence of ^-OH during the electrolysis, which is consistent with the greater stability of $PMn(O_2)^-$ to moisture relative to $PFe(O_2)^-$,[28,29,30] and its larger $(Cl_8TPP)Mn\text{-}OO^-$ bond energy. A species analogous to



$(Cl_8TPP)Fe\begin{smallmatrix}\nearrow OH\\ \searrow OO^-\end{smallmatrix}$ is not generated when $(Cl_8TPP)Mn$, ^-OH, and O_2 are combined despite the favorable stabilization (17 kcal) that results from $(Cl_8TPP)Mn\text{-}OO^-$ formation. This probably is due to the less negative reduction potential for the $(Cl_8TPP)Mn^-OH/(Cl_8TPP)Mn(^-OH)^-$ couple [— 0.43 V versus — 0.70 V for $(Cl_8TPP)Fe\text{-}OH$].

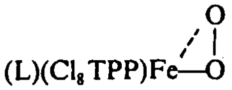
Because the formal reduction potential for the $(Cl_8TPP)Co/(Cl_8TPP)Co^-$ couple, is more positive than that for the $O_2/O_2^{-\cdot}$ couple, direct electrolytic reduction of $[PCo + O_2]$ cannot be applied. However, the $(Cl_8TPP)Co(O_2)^-$ species is generated at the electrode surface when a negative voltage scan is applied to a solution that contains $(Cl_8TPP)Co$, ^-OH, and O_2. The reduction products, $O_2^{-\cdot}$ and $(Cl_8TPP)Co^-$ (E_{red} < —1.4 V), yield $(Cl_8TPP)Co(O_2)^-$ upon reoxidation. The weak $(Cl_8TPP)Co\text{-}OO^-$ bond (8 kcal) necessitates the presence of ^-OH to preclude decomposition via hydrolysis (Eq. (8)). The formal reduction potential for the $(Cl_8TPP)Co\text{-}OH/(Cl_8TPP)Co(^-OH)^-$ couple and the small $PCo\text{-}OO^-$ bond energy preclude



$(Cl_8TPP)Co\begin{smallmatrix}\nearrow OH\\ \searrow OO^-\end{smallmatrix}$ formation.

O_2 Adduct

The electrochemistry (Figure 1) and spectroscopy for the combination of O_2 and metalloporphyrins indicate the absence of interaction between O_2 and $(Cl_8TPP)Zn$, $(Cl_8TPP)Mn$, and $(Cl_8TPP)Co$. However, binding of O_2 to $(Cl_8TPP)Fe$ is indicated by the positive shift of the potential for O_2 reduction (Figure 1c) and the small positive shift for $(Cl_8TPP)Fe$ oxidation in the presence of O_2. These results confirm that formation of $(Cl_8TPP)Fe(O_2)$ is not accompanied by electron transfer from $(Cl_8TPP)Fe$ to O_2. Furthermore, the reduction potential for the $O_2/O_2^{-\cdot}$ couple is more negative than that for the $(Cl_8TPP)Fe^+/(Cl_8TPP)Fe$ couple ($O_2^{-\cdot}$ is used to reduce PFe^+ to PFe). The O-O bond lengths and O-O vibrational frequencies for $PFe(O_2)$ and oxyhemoglobin can be rationalized by arguments that are similar to those presented for the bonding of $(Cl_8TPP)FeOO^-$. Thus, the two unpaired electrons of $^3O_2$ couple with two of the four unpaired *d* electrons of $(Cl_8TPP)Fe$ (d^6sp, $S = 4/2$)



to form two weak covalent bonds and give $(L)(Cl_8TPP)Fe\overset{O}{\underset{}{\diagup}}\!\!|\!O$ (d^6 *sp*, $S = 0; L = \mathrm{DMF}$).

The irreversible reduction of O_2 in the presence of $(Cl_8TPP)Mn$ and the cement-enhancement for the oxidation of $(Cl_8TPP)Mn$ on the reverse scan are consistent with $(Cl_8TPP)MnOO^-$ formation from ($PMn + O_2^{-\cdot}$). The small current increase for the $(Cl_8TPP)Co/(Cl_8TPP)Co^-$ couple in the presence of O_2 appears to be due to the reoxidation of $(Cl_8TPP)Co^-$ by O_2.

The electrochemical, spectroscopic, and magnetic results for the $\cdot OH$, $O_2^{-\cdot}$, and O_2 adducts of $(Cl_8TPP)M$ (M = Fe, Mn, Co) are consistent with the conclusions that the site of electron-transfer is oxygen-centered, and that the oxygen species are stabilized by covalent-bond formation between an unpaired d electron of the transition-metal and an upaired p electron of oxygen. The electrochemistry and magnetic moment for $(Cl_8TPP)FeOO^-$ indicate that the valence electrons of the iron-center are hydridized to d^5sp^2. The formation of a covalent bond between an iron atom and an oxygen species ($O_2^{-\cdot}$) is analogous to such bond formation between a hydrogen atom and an oxygen species to give $H\text{-}OO^-$. The concept of valence-electron hybridization to achieve more effective bonding with iron is equivalent to that for the carbon atom ($s^2p^2 \rightarrow sp^3$).

The present study provides insight to the mechanisms for superoxide-ion disproportionation that are catalyzed by the iron and manganese superoxide-dismutase proteins. The combination of PFe of PMn with $O_2^{-\cdot}$ does not result in electron transfer from the metal; instead they couple to form $PFeOO^-$ and $PMnOO^-$. The latter abstract protons from the medium, and the PFeOOH and PMnOOH products react with a second $O_2^{-\cdot}$ and a proton to give HOOH and O_2 (eq. (9)).

$$\begin{tikzcd}
PFe + O_2^{-\cdot} + HA \arrow[r] & PFe{-}OOH + A^- \arrow[d, "{O_2^{-\cdot},\ HA}"] \\
 & PFe + O_2 + HOOH + A^-
\end{tikzcd} \tag{9}$$

Thus, PFe and PMn facilitate the disproportionation of $O_2^{-\cdot}$, which is equivalent to the function of the iron and manganese superoxide-dismutase proteins. Whether the mechanism suggested by Eq. (9) is relevant to those for the proteins is unkown, but the absence of electron transfer from their metal centers to $O_2^{-\cdot}$ is a reasonable expectation.

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References

1. D.T. Sawyer and J.S. Valentine (1981) How super is superoxide? *Accounts of Chemical Research*, **14**, 393–400.
2. M.E. McAdam, R.A. Fox, F. Lavelle and E.M. Fielden (1977) A pulse-radiolysis study of the manganese-containing superoxide dismutase from Bacillus Stearothermophilus. *Biochemical Journal*, **165**, 71–74.

3. C. Bull and J.A. Fee (1985) Steady-state kinetic studies of superoxide dismutases: Properties of the iron containing protein *Escherichia coli*. *Journal of the American Chemical Society*, **107**, 3295–3304.
4. J.K. Howie and D.T. Sawyer (1976) Manganese(II) and manganese(III) 8-quinolinol complexes. Redox model for mitochondrial superoxide dismutase. *Journal of the American Chemical Society*, **98**, 6698–6700.
5. B.H.J. Bielski and G.G. Shiue (1979) Oxygen free radicals and tissue damage. In *Ciba Foundation Symposium 65*, Excerpta Medica, Amsterdam, pp. 43–56.
6. D.T. Sawyer, L. Spencer, K. Yamaguchi and M.S. McDowell (1986) Tris(picolinato)manganese(II): A chemical model for the mechanism and function of mitochondrial superoxide dismutase. In *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine* (ed. G. Rotilio), Elsevier, New York, pp. 219–224.
7. D.T. Sawyer and J.L. Roberts, Jr. (1974) *Experimental Electrochemistry for Chemists*; Wiley, New York, pp. 44–46, 144–145, 336–339.
8. D.T. Sawyer, T.S. Calderwood, K. Yamaguchi and C.T. Angelis (1983) Synthesis and characterization of tetramethylammonium superoxide. *Inorganic Chemistry*, **22**, 2577–2583.
9. K. Yamaguchi, T.S. Calderwood and D.T. Sawyer (1986) Corrections and additional insights to the synthesis and characterization of tetramethylammonium superoxide [(Me$_4$N)O$_2$]. *Inorganic Chemistry*, **25**, 1289–1290.
10. G.M. Badger, R.A. Jones and R.L. Laslett (1964) Porphyrins: The synthesis of porphyrins by the Rothemund reaction. *Australian Journal of Chemistry*, **17**, 1028–1035.
11. H. Kobayashi, T. Higuchi, Y. Kaizu, H. Osada and M. Aoki (1975) Electronic spectra of tetraphenylporphinatoiron(III) methoxide. *Bulletin of the Chemical Society of Japan*, **48**, 3137–3141.
12. A.D. Alder, F.R. Longo and V. Varadi (1976) Compounds of biological interest (57. Metalloporphrins). *Inorganic Synthesis*, **16**, 213–220.
13. P.S. Traylor, D. Dolphin and T.G. Traylor (1984) Sterically protected hemins with electronegative substituents: Efficient catalysts for hydroxylation and epoxidation. *Journal of Chemical Society, Chemical Communication*, 279–280.
14. C.L. Hill and M.M. Williamson (1985) Structural and electronic properties of six-coordinate Manganese(III) porphyrin cations. Crystal and molecular structure of bis(N,N-dimethylformamide)-(tetraphenylporphinato)-manganese(III) perchlorate, [Mn^{III}TPP(DMF)$_2$]$^+$ ClO$_4^-$ *Inorganic Chemistry*, **24**, 2836–2841.
15. P.K.S. Tsang and D.T. Sawyer (1990) Electron-transfer thermodynamics and bonding for the superoxide (O$_2^{-\cdot}$), dioxygen (·O$_2$·), and (·OH) adducts of tetrakis(2,6-dichlorophenyl)-porphinato-iron, -manganese, and -cobalt in dimethylformamide. *Inorganic Chemistry*, **29**, in press.
16. P.K.S. Tsang, P. Cofré and D.T. Sawyer (1987) Electrochemical oxidation of hydroxide ion in acetonitrile and its facilitation by transition-metal complexes. *Inorganic Chemistry*, **26**, 3604–3609.
17. D.T. Sawyer, G.S. Srivatsa, M.E. Bodini, W.P. Schaefer and R.M. Wing (1986) Redox chemistry and spectroscopy of toluene-3,4-dithiol (TDTH$_2$) and of its M(TDT)$_2^{2-}$/$^-$ complexes with zinc(II), copper(II), nickel(II), cobalt(II), iron(II), and manganese(II); formation of a stable d^n-(·SR) bond upon oxidation by one electron. *Journal of the American Chemical Society*, **108**, 936–942.
18. K. Shin, S.K. Kramer and H.M. Goff (1987) Base-promoted authoreduction of iron(III) porphyrins in dimethyl sulfoxide solution: Magnetic resonance spectroscopy of hydroxoiron(II) porphyrin complexes. *Inorganic Chemistry*, **26**, 4103–4106.
19. G.S. Srivatsa and D.T. Sawyer (1985) Hydroxide-induced reduction of (tetraphenylporphinato) iron(III) in pyridine. *Inorganic Chemistry*, **24**, 1732–1734.
20. J.F. Harrison (1986) The electronic and geometric structures of the chromium cations CrF$^+$, CrO$^+$, CrN$^+$, and CrC$^+$. *Journal of Physical Chemistry*, **90**, 3313–3319.
21. R.T. Sanderson (1986) Electronegativity and bonding of transitional elements. *Inorganic Chemistry*, **25**, 3518–3522.
22. S. Di Bella, I. Fragala and G. Granozzi (1986) Electronic structure of transition-metal tetracoordinated complexes. 1. Theoretical ab initio and uv-photoelectron spectroscopy study of palladium(II) and platinum(II) square-planar acetylacetonate complexes. *Inorganic Chemistry*, **25**, 3997–4003.
23. J.J. Low and W.A. Goddard, III (1986) theoretical studies of oxidative addition and reductive elimination. 3. C-H and C-C reductive coupling from palladium and platinum bis(phosphine) complexes. *Journal of the American Chemical Society*, **108**, 6115–6128.
24. D.T. Sawyer (1987) The nature of the bonding and valency for oxygen in its metal compounds. *Comments on Inorganic Chemistry*, **6**, 103–121.
25. S.A. Richert, P.K.S. Tsang and D.T. Sawyer (1988) Ligand-centered oxidation of manganese(II) complexes. *Inorganic Chemistry*, **27**, 1814–1818.

26. D.T. Sawyer (1990) Electron-transfer thermodynamics, valence-electron hybridization and bonding for iron compounds. *Comments on Inorganic Chemistry*, **10**, 129–159.
27. S.A. Richert, P.K.S. Tsang and D.T. Sawyer (1989) Ligand-centered redox processes for MnL_3, FeL_3 and CoL_3 complexes (L = acetylacetonate, 8-quinolinate, picolinate, 2,2′-bipyridyl, 1,10-phenanthroline) and for their tetrakis (2,6-dichlorophenyl)porphinato complexes [M(por)]. *Inorganic Chemistry*, **28**, 2471–2475.
28. A. Shirazi and H.M. Goff (1982) Characterization of superoxide-metalloporphyrin reaction products: Effective use of deuterium nmr spectroscopy. *Journal of the American Chemical Society*, **104**, 6318–6322.
29. J.N. Burstyn, J.A. Roe, A.R. Miksztal, B.A. Shaevity, G. Lang and J.S. Valentine (1988) Magnetic and spectroscopic characterization of an iron porphyrin peroxide complex. Peroxoferrioctaethylporphyrin(1—). *Journal of the American Chemical Society*, **110**, 1382–1388.
30. R.B. VanAtta, C.E. Strouse, L.K. Hanson and J.S. Valentine (1987) [Peroxotetraphenylporphinato]manganese(III) and [chlorotetraphenylporphinato]manganese(II) anions. Syntheses, crystal structures, and electronic structures. *Journal of the American Chemical Society*, **109**, 1425–1434.
31. E. McCandlish, A.R. Miksztal, M.Nappa, A.Q. Sprenger, J.S. Valentine, J.D. Strong and T.G. Spiro (1980) Reactions of superoxide with iron porphyrins in aprotic solvents. A high spin ferric porphyrin peroxo complex. *Journal of the American Chemical Society*, **102**, 4268–4270.
32. A.D. Goolsby and D.T. Sawyer (1968) The electrochemical reduction of superoxide ion and oxidation of hydroxide ion in dimethyl sulfoxide. *Analytical Chemistry*, **40**, 83–86.
33. J.L. Roberts, Jr., M.M. Morrison and D.T. Sawyer (1978) Base-induced generation of superoxide ion and hydroxyl radical from hydrogen peroxide. *Journal of the American Chemical Society*, **100**, 329–330.
34. Y. Sawaki and Y. Ogata (1981) Mechanism of the reaction of nitriles with alkaline hydrogen peroxide. Reactivity of peroxycarboximidic acid and application to superoxide ion reaction. *Bulletin of the Chemical Society of Japan*, **54**, 793–799.
35. J.S. Valentine and A.E. Quinn (1976) Reaction of superoxide with the manganese(III) tetraphenylporphine cation. *Inorganic Chemistry*, **15**, 1977–1999.

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