A Seven-Coordinate Manganese(II) Complex Formed with a Single Tripodal Heptadentate Ligand as a New Superoxide Scavenger

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Abstract: A new manganese(II) complex, Mn(II)−tris[2-[*N*-(2-pyridylmethyl)amino]ethyl]bishexafluorophosphate (Mn−TPAA), has been synthesized, its X-ray crystal structure resolved, and its superoxide stoichiometric scavenging activity established. The complex has been formed with the tripodal potentially heptadentate ligand TPAA which turns out to be the single ligand coordinated to the metal ion and therefore achieves the seven coordination. The complex crystallizes in the space group $P2_12_12_1$, $a = 19.654(9)$ Å, $b = 15.416(5)$ Å, $c = 10.425(4)$ Å, with four formula units per unit cell. The manganese is bonded to three pyridine nitrogen atoms, to three amine groups, and to the tripodal bridging nitrogen N. The reactivity toward superoxide has been investigated using the indirect xanthine−xanthine oxidase−cytochrome *c* method, the electrochemical reaction of *in-situ* generated superoxide, and γ and pulse radiolysis measurements. From the cytochrome *c* assay, it has been found that the IC_{50} value is equal to 4.4 μM. From electrochemical experiments performed in dry acetonitrile and in the presence of oxygen, the complex appears to induce the one-electron reduction of oxygen to split into two separate steps. Electrochemistry also shows that superoxide reacts with the complex. Confirmation of this reaction is obtained with pulse radiolysis which allows the observation of a transient, visualized by its difference absorption spectrum, with a rate constant of 1.05×10^7 M^{-1} s^{-1}. Inhibition of the formation of H_2O_2 is also found.

Introduction

Superoxide dismutases (SOD)[1] are metalloenzymes that catalyze the dismutation of superoxide anion (O_2^-) to hydrogen peroxide and dioxygen. They constitute an important defense system in living organisms against several diseases in which O_2^- appears to play an important role. For example, superoxide is supposed to be implicated as a cause of tissue inflammation, symptoms of aging, some cancers, and the cellular degenerating process promoted by AIDS.[2] The direct utilization of the enzymes as pharmaceutical agents[3] poses many problems. So considerable efforts have been made in order to obtain stable, nontoxic, and inexpensive low molecular weight biomimetic molecules which are able to catalyze superoxide dismutation and therefore to provide important therapeutic applications. Many systems have been isolated, displaying a superoxide scavenger activity, and have been proposed as SOD models. Most of them are copper complexes[4] which have been identified as (Cu−Zn)SOD mimics. There are some reports on iron[5]- or manganese[6]-containing molecules presented as FeSOD or Mn-SOD models. SOD-like activity of these systems is still a controversial problem, due to possible uncertainties in using indirect methods to quantify superoxide involved in the reaction.[7] Recently, a family of manganese macrocyclic ligand complexes has been screened for SOD activity using direct

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(1) (a) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049. For a review, see for example: (b) Michelson, A. M.; McCord, J. M.; Fridovich, I. *Superoxide and Superoxide Dismutases*; Academic Press: New York, 1977. (c) Fee, J. A. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker, Inc.: New York, 1981; p 259. (d) Valentine, J. S.; Pantoliano, M. W. In *Copper Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1981; p 291. (e) Fridovich, I. *Annu. Rev. Biochem.* **1995**, *64*, 113.

(2) (a) Halliwell, B.; Gutteridge, J. M. C. Free radicals, ageing and disease. In *Free Radicals in Biology and Medicine*, 2nd ed.; Clarendon Press: Oxford, 1989. (b) Sinha, B. K.; Mimnaugh, E. G. *Free Radical Biol. Med.* **1990**, *8*, 567. (c) Sun, Y. *Free Radical Biol. Med.* **1990**, *8*, 583. (d) Barnes, P. J. *Free Radical Biol. Med.* **1990**, *9*, 235. (e) Halliwell, B.; Gutteridge, J. M. C.; Cross, C. E. *J. Lab. Clin. Med.* **1992**, *119*, 598. (f) Schreck, R.; Richer, P.; Baewerle, P. A. *EMBO J.* **1991**, *10*, 2247.

(3) (a) Bolli, R. *Cardiovasc. Drugs Ther.* **1991**, *5*, 249. (b) Engler, R., Gilpin, E. *Circulation* **1989**, *79*, 1137. (c) Johanson, M. H.; Deinum, J.; Marklund, S. L.; Sjoquist, P. *Cardiovasc. Res.* **1990**, *24*, 500.

(4) (a) O'Young, C. L.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 4920−4924. (b) Kimura, E.; Sakonaka, A.; Nakamoto, M. *Biochim. Biophys. Acta* **1981**, *678*, 172. (c) Kimura, E.; Koike, T.; Shimizu, Y.; Kodama, M. *Inorg. Chem.* **1986**, *25*, 2242. (d) Pierre, J. L.; Chautemps, P.; Refaif, S. M.; Beguin, C. G.; El-Marzouki, A.; Serratrice, G.; Saint-Aman, E.; Rey, P. *J. Am. Chem. Soc.* **1995**, *117*, 1965−1973.

(5) (a) Bull, C.; McClune, G. J.; Fee, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 5290. (b) Nagano, T.; Hirano, T.; Hirobe, M. *Free Radical Res. Commun.* **1991**, *12*, 221. (c) Iuliano, L.; Pedersen, J. Z.; Ghiselli, A.; Pratico, D.; Rotilio, G.; Violi, F. *Arch. Biochem. Biophys.* **1992**, *293*, 153. (d) Farragi, M.; Peretz, P.; Weinraub, D. *Int. J. Radiat. Biol.* **1986**, *49*, 951.

(6) (a) Stein, J.; Fackler, J. P., Jr.; McClure, G. J.; Fee, J. A.; Chan, L. T. *Inorg. Chem.* **1979**, *18*, 3511. (b) Darr, D. L.; Yanni, S.; Pinnell, S. J. *Free Radical Biol. Med.* **1988**, *4*, 357. (c) Kitajima, N.; Osawa, M.; Tamura, N.; Moro-oka, Y.; Hirano, T.; Hirobe, M.; Nagano, T. *Inorg. Chem.* **1993**, *32*, 1879. (d) Hahn, S. M.; Krishna, C. M.; Samuni, A.; Mitchell, J. B.; Russo, A. *Arch. Biochem. Biophys.* **1991**, *288*, 215. (e) Faulkner, K. M.; Stevens, R. D.; Fridovich, I. *Arch. Biochem. Biophys.* **1994**, *310*, 341 and references therein.

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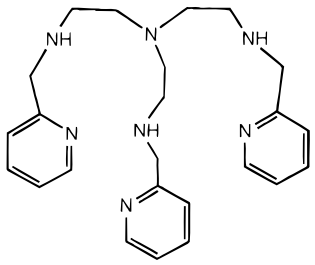
Chart 1. TPAA



quantitative stopped-flow analysis,[7c] and two of them have been demonstrated to be efficient SOD mimics[8] with high activity. In most cases, the catalytic mechanism is not clearly established because identification of transient species has been difficult.

In order to gain more insight into the reactivity of transition metal complexes with superoxide, we have initiated a program to investigate new iron- or manganese-containing molecules[9] either with new tetradentate biomimetic ligands which could mimic the endogeneous structural features of the active sites[10] or with multidentate hindered polyamine ligands which demonstrate some similarities with oligopeptide complexes as has been proposed, for example, in the case of macrocyclic polyamine molecules.[4b] In addition to this interest in the development of SOD mimics as putative pharmaceutical agents, there is also considerable interest in expanding the chemistry of these systems which might be involved in activation of dioxygen and hydrogen peroxide, and currently the subject of intensive work.[11]

In the literature, the potentially heptadentate tripodal ligand TPAA (TPAA = Tris[2-[*N*-(2-pyridylmethyl)amino]ethyl]amine ligand, represented in Chart 1) was supposed to chelate iron(III). The corresponding complex was reported as one of the most active SOD mimics.[7b,12,13] These studies require a better understanding of the chemistry of TPAA-containing molecules since neither structural nor physicochemical characteristics were investigated. They have led us to investigate possible manganese(II) chelation, manganese being less toxic for organisms than iron. We now report on a new TPAA−manganese(II) complex (abbreviated Mn−TPAA) which has been structurally characterized as a seven-coordinate manganese complex, with the coordination being achieved by only one ligand. Its reactivity toward superoxide has been proved through spectroscopic, electrochemical, and γ and pulse radiolysis studies. An oxygenated transient species has been discovered.

Table 1. Crystallographic Data for [Mn−TPAA](PF$_6$)$_2$

formula	$C_{24}H_{33}N_7MnP_2F_{12}$
mol wt	764.45
a, Å	19.654(9)
b, Å	15.416(5)
c, Å	10.425(4)
$\alpha = \beta = \gamma$, deg	90.00
V, Å^3	3159(2)
Z	4
space group	$P2_12_12_1$, orthorhombic
D_c, g cm^{-3}	1.608
radiation (λ, Å)	Mo Kα (0.710 73)
temp, K	294
abs coeff, mm^{-1}	0.619
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.053$, $wR2 = 0.132$
R indices (all data)	$R_1 = 0.087$, $wR2 = 0.176$
no. of reflections	4231
no. of parameters	415

Experimental Section

Materials and Methods. Commercial reagents were used as obtained without further purification. Solvents were either of reagent or spectroscopic grade and were purified by conventional procedures prior to use. Elemental analyses were performed by the CNRS Microanalysis Laboratories of Gif sur Yvette and Lyon, France.

Synthesis of the Ligand TPAA. TPAA[12] was prepared by a novel experimental procedure. To a stirred solution of tris(2-aminoethyl)-amine (2.92 g, 20 mmol) in 60 mL of dry methanol was added 2-pyridinecarboxaldehyde (6.4 g, 60 mmol). The stirred mixture was hydrogenated for 12 h under atmospheric pressure and at room temperature in the presence of 1 g of palladium on activated carbon (10% Pd). Then the catalyst was filtered off and washed with 15 mL of dry methanol. The filtrate and washings were collected, evaporated, and dried under vacuum, yielding 7.5 g of a yellow-brown oil (89%). The ligand was used without any further purification. ^{1}H NMR in $CDCl_3$ (δ in ppm): 2.62 (t, 6H, CH_2CH_2N), 2.69 (t, 6H, NCH_2CH_2), 3.87 (s, 3H, $PyrCH_2N$), 7.09 (m, 3H, Pyr), 7.25 (m, 3H, Pyr), 7.56 (m, 3H, Pyr), 8.44 (m, 3H, Pyr). ^{13}C NMR in $CDCl_3$ (δ in ppm): 45.59 (NCH_2CH_2), 52.08 ($PyrCH_2N$), 52.17 (CH_2CH_2N), 122.10 (Pyr), 122.47 (Pyr), 136.39 (Pyr), 148.48 (Pyr), 155.47 (Pyr). Mass spectrum (CI): m/z = 420 [M + 1]. IR (cm^{-1}, NaCl plate): 3300 (m, ν_{N-H}), 3000−2800 (s or m, ν_{C-H}), 1591, 1570, 1474, 1433 (vs, $\nu_{pyridine\ ring}$).

Synthesis of the Complex Mn−TPAA. To a stirred solution of TPAA (0.3 g, 0.71 mmol) in 15 mL of methanol was slowly added $MnCl_2 \cdot 4H_2O$ (0.140 g, 0.71 mmol) in water (10 mL). The resulting yellow solution was refluxed for 1 h, and then NH_4PF_6 (0.25 g, 1.56 mmol) dissolved in 5 mL of methanol was added to induce precipitation of the complex. The white precipitate was filtered and recrystallized from an ethanol/acetone (1:1) solution by slow evaporation of the solvent at room temperature. After filtration, pale pink crystals of Mn−TPAA were obtained (0.33 g, yield 62%). Anal. Calcd for $C_{24}H_{33}N_7MnP_2F_{12}$: C, 37.70; H, 4.35; N, 12.82; P, 8.11; Mn, 7.18. Found: C, 37.7; H, 4.15; N, 12.55; P, 8.32; Mn, 7.06. The mass spectrum (PDMS ^{252}Cf) displayed a molecular ion peak for [$C_{24}H_{33}N_7Mn$] at m/z = 474.2 [M]$^+$. IR (KBr disk, cm^{-1}): 3325 (s, ν_{N-H}); 2950−2800 (m, ν_{C-H}); 1603, 1571, 1477, 1445 (s, $\nu_{pyridine\ ring}$); 834, 555 (vs, ν_{P-F}). Electronic spectrum in DMSO: λ_{max} (ϵ_M) 264 (10984).

Crystallographic Studies. The synthesis of complex Mn−TPAA afforded well-shaped crystals suitable for X-ray diffraction study. A bright pink prismatic crystal of 0.2 × 0.5 × 0.7 mm was mounted on a four-circle Philips diffractometer equipped with a graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). Accurate cell parameters were obtained from least-squares refinement of 24 independent reflections (9.8° < θ < 10.9°). They are reported in Table 1 with other pertinent details for the structure determination. The data collection was performed at room temperature using the $\theta-2\theta$ scan technique mode (range 2−28°, speed 0.05 deg s^{-1}, scan width 1.0°). Three standard reflections were measured every 180 min, and showed no significant decay. A total of 4252 reflections [0 < h < 25, 0 < k < 20, 0 < l < 13] were collected. From 4251 unique reflections, 4231 were used. These data were corrected for Lorentz and polarization factors but not for absorption.

(7) (a) Bielski, B. H.; Cabelli, D. E. *Int. J. Radiat. Biol.* **1991**, *59*, 291. (b) Weiss, R. H.; Flickinger, A. G.; Rivers, W. J.; Hardy, M. M.; Aston, K. W.; Ryan, U. S.; Riley, D. P. *J. Biol. Chem.* **1993**, *31*, 23049. (c) Riley, D. P.; Rivers, W. J.; Weiss, R. H. *Anal. Biochem.* **1991**, *196*, 344.

(8) Riley, D. P.; Weiss, R. H. *J. Am. Chem. Soc.* **1994**, *116*, 387−388.

(9) Rodriguez, M. C.; Deroche, A.; Morgenstern-Badarau, I. Studies in progress.

(10) (a) Tainer, J. A.; Getzoff, E. D.; Beem, K. M., Richardson, J. S.; Richardson, D. C. *J. Mol. Biol.* **1981**, *160*, 181−217. (b) Stallings, W. C.; Powers, T. B.; Pattridge, K. A.; Fee, J. A.; Ludwig, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3884−3888. (c) Ludwig, M. L.; Metzger, A. L.; Pattridge, K. A.; Stallings, W. C. *J. Mol. Biol.* **1991**, *219*, 335−358. (d) Lah, M. S.; Dixon, M. M.; Pattridge, K. A.; Stallings, W. C.; Fee, J. A.; Ludwig, M. L. *Biochemistry* **1995**, *34*, 1646.

(11) See for examples: (a) Sawyer, D. T.; Kang, C.; Llobet, A.; Redman, C. *J. Am. Chem. Soc.* **1993**, *115*, 5817−5818. (b) Kang, C.; Sobkowiak, A.; Sawyer, D. T. *Inorg. Chem.* **1994**, *33*, 79−82. (c) Kojima, T.; Leising, R. A.; Yan, S.; Que, L., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 9524−9530. (d) For a review, see: Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759−805.

(12) Nagano, T., Hirano, T., Hirobe, M. *J. Biol. Chem.* **1989**, *264*, 9243.

(13) Nagano, T., Hirano, T., Hirobe, M. *Free Radical Res. Commun.* **1991**, *12−13*, 221.

Table 2. Selected Bond Lengths (Å) and Bond Angles (deg) for $[Mn-TPAA](PF_6)_2$

Mn−N(3A)	2.270(5)	Mn−N(6C)	2.380(5)
Mn−N(3B)	2.299(5)	Mn−N(6A)	2.424(5)
Mn−N(3C)	2.306(5)	Mn−N	2.499(4)
Mn−N(6B)	2.367(5)		
N(3A)−Mn−N(3B)	114.4(2)	N(3B)−Mn−N(6A)	160.5(2)
N(3A)−Mn−N(3C)	108.9(2)	N(3C)−Mn−N(6A)	81.5(2)
N(3B)−Mn−N(3C)	112.8(2)	N(6B)−Mn−N(6A)	89.6(2)
N(3A)−Mn−N(6B)	85.8(2)	N(6C)−Mn−N(6A)	85.6(2)
N(3B)−Mn−N(6B)	72.3(2)	N(3A)−Mn−N	73.8(2)
N(3C)−Mn−N(6B)	158.8(2)	N(3B)−Mn−N	72.6(2)
N(3A)−Mn−N(6C)	155.0(2)	N(3C)−Mn−N	73.4(2)
N(3B)−Mn−N(6C)	86.3(2)	N(6B)−Mn−N	126.6(2)
N(3C)−Mn−N(6C)	72.6(2)	N(6C)−Mn−N	128.3(2)
N(6B)−Mn−N(6C)	87.7(2)	N(6A)−Mn−N	125.8(2)
N(3A)−Mn−N(6A)	70.2(2)		

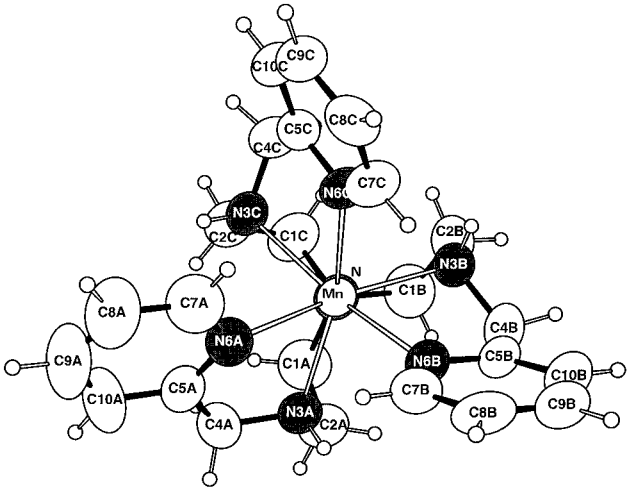


Figure 1. Ortep view of the cation $[Mn-TPAA]^{2+}$ showing 50% probability thermal ellipsoids, and the atom-labeling scheme.

The structure was solved by direct methods (program SHELXS86[14a]) and refined on F^2 for all reflections by least-squares methods, using SHELXL-93.[14b] All hydrogen atoms were located from a difference Fourier map and refined in the rigid model with isotropic thermal factors equal to 1.1 times that of the bonded atom. The final conventional R factor was 0.053 for 2916 $F_o > 4\sigma(F_o)$ and 415 parameters, $wR(F^2) = 0.176$ for all, $S = 1.018$, $w = [\sigma^2(F_o)^2 + (0.0774P)^2 + 1.6641P]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$. The largest difference peak and hole are 0.39 and −0.34 e Å^{-3}.

Crystal data with details of the diffraction experiment and subsequent calculations are summarized in Table 1. Selected bond lengths and angles are listed in Table 2. The ORTEP plot of the cation $[Mn-TPAA]^{2+}$ is shown in Figure 1. A complete listing of bond lengths, bond angles, and anisotropic thermal parameters are supplied as supporting information.

Physical Methods and Reactivity Studies. Electronic absorption spectra were recorded on a Safas 190 DES spectrophotometer at room temperature with quartz cells. Infrared spectra were recorded on a IFS 66 Fourier transform infrared Bruker spectrometer using a KBr pellet for solid samples and NaCl plates for neat liquid samples. ^{1}H and ^{13}C NMR spectra of the ligands were recorded on AM 200 and AM 250 Bruker spectrometers using deutered solvent. Mass spectra in the positive mode were obtained with a time-of-flight mass spectrometer fitted with a ^{252}Cf source. EPR spectra were recorded at room temperature on a Bruker ER 200 D spectrometer operating at 9.4 GHz.

Superoxide was introduced into the system in three different ways, from xanthine−xanthine oxidase reaction, from electrochemical reduction of dioxygen, and from pulse radiolysis experiments, according to the following detailed procedures.

(14) (a) Sheldrick, G. M. SHELXS-86, Göttingen, Germany, 1986. (b) Sheldrick, G. M. SHELXL-93, Göttingen, Germany, 1993.

Xanthine−Xanthine Oxidase−Cytochrome *c* Assay. Superoxide anions were supplied to the evaluating system from the xanthine−xanthine oxidase reaction as described in the literature.[15] The SOD activities were evaluated using the ferricytochrome *c* reduction technique modified from Fridovich.[16] The assay was performed at 25 °C in 3 mL of reaction buffer (50 mM potassium phosphate, pH 7.8) containing 22 μM cytochrome *c*, 100 μM xanthine, 500 U/mL catalase, and an amount of xanthine oxidase sufficient to give an initial rate of $\Delta A_{550\ nm/min} = 0.025$ in the absence of the SOD mimic. Reduction of ferricytochrome *c* was followed at 550 nm, and rates were linear for at least 4 min. The IC_{50} value represents the concentration of the complex that exerts the SOD activity equivalent to one unit of native SOD. The IC_{50} value of the Mn−TPAA complex was determined both with and without addition of bovine serum albumine, 0.5 mg/mL.

Electrochemical Studies. Experiments were performed at room temperature in dry acetonitrile containing 0.1 M $TBAPF_6$ (tetrabutylammonium hexafluorophosphate), using a three-compartment cell. The reference electrode was a saturated calomel electrode (SCE), separated from the test solution by an intermediate bridge containing the supporting electrolyte and closed by a fine porosity ceramic frit to prevent contamination of the test solution by chloride ions and water. The counter electrode was a platinum gauze soaked in the supporting electrolyte and separated from the test solution by a medium porosity glass frit. The working electrode was either a thoroughly-polished 3 mm diameter glassy carbon disk (Tokai, Japan) or a 1 mm diameter Pt disk. The solutions were thoroughly degassed with pure argon prior to admission of small amounts of oxygen in the cell when necessary. The electrochemical setup was an EG&G 273A potentiostat driven by a PC with 270 software.

Pulse Radiolysis. Pulse radiolysis was performed using the setup equipment at the Institut Curie-Biologie, Orsay. This setup has been described previously.[17] The relative dosimetry is obtained using the flash of Cerenkov light, whose integrated intensity is proportional to the number of electrons received in the cell. The calibration was made by comparison with methyl viologen (MV^{2+}) ($[MV^{2+}] = 10$ mM, phosphate buffer (10 mM, pH 8), formate (0.1 M)). Methyl viologen was reduced by CO_2^- free radicals (produced by irradiation of formate-containing solutions in an atmosphere of nitrous oxide), giving MV^+ free radicals stable in the absence of oxygen [$G(MV^+$ radical$) = 0.62$ μmol J^{-1}, observation at 600 nm, $\epsilon_{600\ nm} = 13.600$ M^{-1} cm^{-1}].[18]

For γ as well as for pulse radiolysis experiments, solutions to be irradiated were made up in 1 mM phosphate buffer and contained sodium formate 0.1 M (pH 7.8). The samples were equilibrated with O_2 by flushing for at least 60 min in dim light under permanent stirring or vortexing, thus avoiding bubbling in the solution. It is known that in such conditions O_2^- free radicals are formed by the following reaction sequence:

$$H_2O \xrightarrow{h\nu} OH^\bullet, e_{aq}^-, H^\bullet, H_2O_2, H_2, H_3O^+$$

$$OH^\bullet + HCO_2^- \rightarrow CO_2^- + H_2O$$

$$CO_2^- + O_2 \rightarrow CO_2 + O_2^-$$

$$e_{aq}^- + O_2 \rightarrow O_2^-$$

$$H^\bullet + O_2 \rightarrow HO_2^\bullet$$

$$HO_2^\bullet \rightleftharpoons H^+ + O_2^- \qquad pK_a = 4.7$$

Because of the high rate constants of these reactions, all the primary radicals are converted into O_2^- in less than 1 μs after the pulse with a radiolytic yield of 0.62 μmol J^{-1}. The change in the absorbance of O_2^- was followed at 250 nm.

(15) Fridovich, I. In *CRC Handbook of Methods for Oxygen Radical Reasearch*; Greenwald, R. A., Ed.; CRC: Boca Raton, FL, 1985; p 51.
(16) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049.
(17) Favaudon, V.; Tourbez, H.; Houée-Levin, C.; Lhoste, J. M. *Biochemistry* **1990**, *29*, 10978.

Table 3. Selected $M-N_{tripodal}$ Bond Distances

	$M-N_{tripodal}$, Å	metal ion	coordination number	ref
Complexes with Imine-Type Ligands				
$[Mn-(py)_3tren)](BF_4)_2$	2.794(2)	Mn(II)	pseudo-7	23
$[Co-(py)_3tren)](BF_4)_2$	2.870(2)	Co(II)	pseudo-7	23
$[Zn-(py)_3tren)](BF_4)_2$	3.013(2)	Zn(II)	6	23
$[Cu-(py)_3tren)](BF_4)_2$	3.110(2)	Cu(II)	6	23
$[Mn-(sal)_3tren)]$	3.229(6)	Mn(III)	6	24
$[Fe-(pyrol)_3tren)]$	3.304(1)	Fe(III)	6	25
$[Co-(pyo)_3tren)](PF_4)_2 \cdot CH_3CN$	2.445(11)	Co(II)	7	22
$[Mn-(pyo)_3tren)](PF_6)_2 \cdot C_2H_5OH$	2.483(1)	Mn(II)	7	22
Complexes with Amine-Type Ligands				
$[Mn-TPAA](PF_6)_2$	2.499(4)	Mn(II)	7	this work

γ irradiations were performed with a ^{137}Cs IBL 637 irradiator (Cis-Bio) at a dose rate of 2 Gy min^{-1}. The dosimetry was made using Fricke's procedure.[19] In γ radiolysis, a steady state is created which lasts as long as the irradiation. H_2O_2 was quantified by oxidation of iodide using the Ghormley procedure.[20]

Results and Discussion

Preparation and Characterization of Mn−TPAA. A three-step synthesis of the free ligand TPAA was previously reported.[12] We have developed a new procedure to synthesize the ligand in one step without need of a purification step. The ligand was obtained with good yield (89%). The complex was prepared by addition of a stoichiometric amount of $MnCl_2 \cdot 4H_2O$ in water to a solution of TPAA in methanol. Then, Mn−TPAA was precipitated by addition of NH_4PF_6 and recrystallized from an ethanol/acetone solution. Mn−TPAA is pale pink and was characterized by elemental analysis, infrared spectroscopy, and PDMS ^{252}Cf mass spectrometry. Mn−TPAA is soluble in DMSO, CH_3CN, and methanol and slightly soluble in water. The UV−vis spectrum in DMSO shows only a very intense absorption at 264 nm ($\epsilon = 10\,980\ M^{-1}\ cm^{-1}$) probably due to an internal pyridine $\pi-\pi^*$ transition. A similar UV−vis spectrum is obtained in aqueous solution and remains unchanged in the pH range 5−9.5. The X-band EPR spectrum of Mn−TPAA dissolved in DMSO at room temperature shows the typical six-line hyperfine signal centered at $g = 2$ which is associated with the $I = 5/2$ nuclear spin of ^{55}Mn. The experimental hyperfine coupling constant is equal to 93 G and is of the same order as that found for other mononuclear Mn(II) complexes.[21] This spectrum confirms that the material is high-spin Mn(II). In the solid state at 300 K, a complicated, unsymmetrical multiple signal spectrum centered at $g = 2$ is observed without apparent hyperfine splitting. Similar spectra have been reported for monomeric Mn(II) complexes in the solid state.[21] The many features of the spectrum indicate a low ligand-field symmetry, possible interactions between manganese centers, and, most importantly, a weak zero-field splitting ($D < h\nu$).[21c,d]

Molecular Structure of Mn−TPAA. An ORTEP representation of the cation of Mn−TPAA, showing the atomic numbering, is presented in Figure 1. The complex crystallizes in the orthorhombic space group $P2_12_12_1$. The manganese is heptacoordinated by seven nitrogen atoms from TPAA, the coordination polyhedron being best described as a monocapped antitrigonal prism of [MnN7], in which the tripodal N atom is bonded to the metal atom. Three nitrogen atoms of the CH_2-NH bonds and three nitrogen atoms of the pyridine ring form two ideal equilateral triangles between which the manganese atom is located. The two triangles, which are parallel, are mutually staggered by $\Phi = 54.21°$, so the geometry is twisted from ideal antitrigonal ($\Phi = 60°$) by 6° toward trigonal prismatic. The complex cation symmetry is close to C3, the C3 axis passing through the manganese and the N tripodal atom (Figure 1). However, one of the arms, labeled A, adopts a slightly different conformation due to interactions with other molecules. The average $Mn-N_{amine}$ and $Mn-N_{pyridine}$ bond distances are 2.28 and 2.38 Å, respectively. The $Mn-N_{tripodal}$ bond distance, equal to 2.49 Å, is slightly longer than the other Mn−N distances but shorter than the sum of the van der Waals radii (2.82 Å). The formation of this bond induces some strains in the molecule; thus, the angles between the three secondary amines N3 are quite enlarged (mean value 112°) with respect to octahedral geometry (90°). In contrast, the angles between the pyridine nitrogens N6 are slightly smaller (mean value 88°). Many tripodal potentially heptadentate ligands have been reported.[22−25] All of them are imine-type ligands resulting from the condensation of various aldehydes and tren.[26] The $Mn-N_{tripodal}$ bond distance found in Mn−TPAA is close to the $M-N_{tripodal}$ distances observed in two heptacoordinated complexes[22] containing the tripodal ligand $(pyo)_3tren$ (Table 3). Nevertheless, most of the imine-type ligands form hexacoordinated metal complexes with long $M-N_{tripodal}$ distances (Table 3). It has been reported that the $[Mn-(py)_3tren)]^{2+}$ complex,[23] with a ligand similar to TPAA but containing CH=N bonds instead of CH_2-NH, exhibits a $Mn-N_{tripodal}$ distance equal to 2.79 Å, and is only described as "pseudoheptacoordinated" (Table 3). This difference is certainly due to a stronger chelating effect of the α-diimine linkage present in $(py)_3tren$. All these results are in agreement with the formation of a coordinate bond between the $N_{tripodal}$ atom and manganese, and confirm the heptacoordination of Mn−TPAA.

Heptacoordination occupies a special niche in coordination chemistry and throughout the periodic table, structurally characterized examples of heptadentate ligands coordinating one

(18) Mulazzani, Q. G.; D'Angelantonio, M.; Venturi, M.; Hoffman, M. Z.; Rogers, M. A. *J. Phys. Chem.* **1986**, *90*, 5347−5352.
(19) Spinks, J. W. T.; Woods, R. J. *Introduction to Radiation Chemistry*; J. Wiley: New York, 1990; see also references therein.
(20) Allen, A. O., Hochanadel, C. J.; Ghormley, J. A.; Davies, T. W. *J. Phys. Chem.* **1952**, *56*, 575.
(21) (a) Kessissoglou, D. P.; Butler, W. M.; Pecoraro, V. L. *Inorg. Chem.* **1987**, *26*, 495. (b) Morrison, M. M.; Sawyer, D. T. *Inorg. Chem.* **1978**, *17*, 333. (c) Mabad, B.; Cassoux, P.; Tuchagues, J.-P.; Hendrickson, D. N. *Inorg. Chem.* **1986**, *25*, 1420. (d) Flassbeck, C.; Wieghartd, K.; Bill, E.; Butzlaff, C.; Trautwein, A. X.; Nuber, B.; Weiss, J. *Inorg. Chem.* **1992**, *31*, 21.
(22) Gou, S.; You, X.; Yu, K., Lu, J. *Inorg. Chem.* **1993**, *32*, 1883.
(23) Kichner, R. M.; Mealli, C.; Bailey, M.; Howe, N.; Torre, L. P.; Wilson, L. J.; Andrews, L. C.; Rose, N. J.; Lingafelter, E. C. *Coord. Chem. Rev.* **1987**, *77*, 89.
(24) Alcock, N. W.; Cook, D. F.; McKenzie, E. D.; Worthington, J. M. *Inorg. Chim. Acta* **1980**, *38*, 107.
(25) Sim, P. G., Sinn, E. *Inorg. Chem.* **1978**, *17*, 1288.
(26) Abbreviations used: tren = tris(2-aminoethyl)amine; py = 2-pyridinecarboxaldehyde; pyo = 2-pyridinecarboxaldehyde *N*-oxide, sal = 5-chlorosalicylaldehyde, pyrol = pyrrole-2-carboxaldehyde.

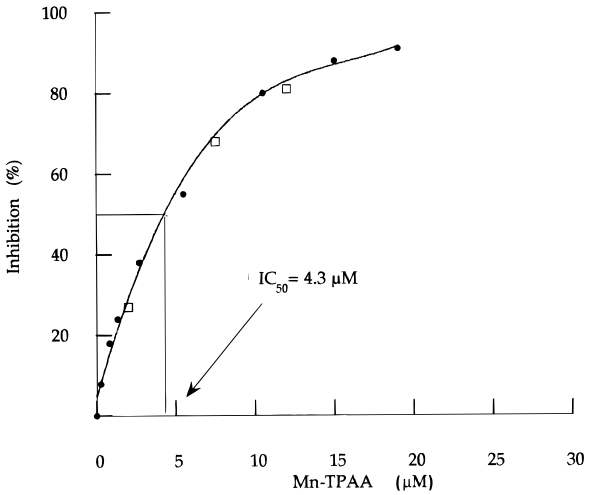


Figure 2. Effect of Mn−TPAA on the inhibition of ferricytochrome *c* reduction by xanthine−xanthine oxidase-generated superoxide. Conditions: potassium phosphate buffer, 50 μM (pH 7.8); 22 μM cythochrome *c*; 100 μM xanthine; catalase, 500 U/mL; Mn−TPAA at the indicated concentrations in buffer; an amount of xanthine oxidase such that an initial rate of $\Delta A_{550\ nm/min} = 0.025$ was obtained. Key: ●, activity of Mn−TPAA; □, activity of Mn−TPAA in presence of 0.5 mg/mL bovine serum albumin.

metal ion being rare.[27] In nearly all cases, seven-coordination results from pentadentate ligands occupying five coordination sites together with two axial ligands.[28] To our knowledge, there are only two other examples of manganese complexes heptacoordinated by only one ligand: a Mn(II) complex with a unit of N7 containing a [21ane N7] polyazamacrocyclic ligand[29] and the [Mn(II)−(pyo)$_3$tren] complex reported in Table 3.

Reactivity with Superoxide. The reactivity of Mn−TPAA toward superoxide has been investigated using the indirect method of xanthine−xanthine oxidase−ferricytochrome *c* assay and direct methods such as the electrochemical reaction of *in-situ* generated superoxide, and γ and pulse radiolysis in a formate−oxygen aqueous solution.

Xanthine−Xanthine Oxidase−Ferricytochrome *c* Assay. We have controlled different parameters to improve the reliability of the test. To examine whether the putative superoxide scavenger was active in the cytochrome *c* assay as a result of the inhibition of xanthine oxidase activity, the conversion of xanthine to urate was determined by measuring the increase in absorbance at 290 nm over a period of 3 min in the presence and absence of the test compound. At concentrations higher than its IC_{50} value, Mn−TPAA did not inhibit this conversion. Catalase (500 U/mL) was added in the assay to be certain that the inhibitory effect observed was not due to oxidation of reduced cytochrome *c* by hydrogen peroxide and complex-dependent reaction.[7] From the assay, we have found that Mn−TPAA inhibits the reduction of ferricytochrome *c* by enzymatic flux of superoxide. The IC_{50} value (Figure 2) is 4.3 μM. It has been reported that bovine serum albumin (BSA), a powerful biological chelating agent, suppressed SOD-like activities of many copper models.[12] The inhibitory effect of Mn−TPAA was tested in the presence of 0.5 mg/mL BSA. We found that

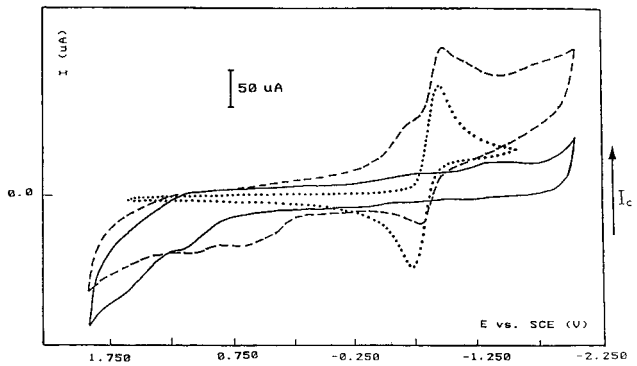


Figure 3. Cyclic voltammograms of acetonitrile solution containing 0.1 M $TBAPF_6$ and various substrates (scan rate 200 mV/s): (a) —, 2 mM Mn−TPAA; (b) - - -, 2 mM Mn−TPAA + 3 mM oxygen; (c) ···, 3 mM oxygen.

BSA has no effect on the activity of Mn−TPAA (Figure 2). This suggests that the complex might remain intact within cells and might retain reactivity *in vivo*. By comparison with other Mn−SOD mimics, Mn−TPAA activity measured by the cytochrome *c* assay is of the same order of magnitude as the activity of Mn−Desferal[6d,e] or Mn−EDTA.[6a] To date, the best activity was found with a (benzoato)manganese(II) complex[6c] that exhibits an $IC_{50} = 0.7$ μM.

Very often, the SOD-like activity of model complexes is measured using the cytochrome *c* assay. However, the determination of catalytic activity using this test is very controversial, and it has been reported that such indirect assays can give false positives for SOD activities.[7] Thus, it has been recently established by stopped-flow kinetic analysis that Mn−Desferal and Mn−EDTA do not catalyze the dismutation of superoxide.[7b] Therefore, direct monitoring of superoxide decay is necessary to obtain accurate and reliable activity measurements.

Electrochemical Studies. Figure 3 shows typical cyclic voltammograms recorded with a glassy carbon working electrode and illustrates the behavior of the present system. The voltammogram in Figure 3a consists of a series of small and ill-defined waves in the anodic as well as in the cathodic domains for the Mn(II) complex. The pattern is not significantly improved with a Pt working electrode. Even though some electroactivity of the complex solution is thus detected, it seems difficult to ascribe unequivocally any of the waves to metal rather than to ligand-centered electron transfer. Such a behavior of Mn(II) complexes is not uncommon.[30a] However, a remarkable pattern is observed in the presence of oxygen as shown in Figure 3b. Figure 3c shows the cyclic voltammogram of oxygen in the absence of Mn−TPAA. We note that the superoxide anion is perfectly stable in the supporting electrolyte alone, at least on the voltammetric time scale, which underscores the dryness of the medium. By comparison of parts b and c of Figure 3, it appears that the presence of Mn−TPAA produces a new reduction wave at a more positive potential than that of the oxygen reduction wave. This new wave is chemically irreversible. Concomitantly, the anodic wave corresponding to the oxidation of O_2^- is diminished and can vanish completely when the concentration of Mn−TPAA is increased for a fixed concentration of dioxygen. It should be noted that oxygen can be excluded completely from the solution by an argon stream, and the situation observed in Figure 3a is restored. This result

(27) (a) Drew, M. G. B. *Prog. Inorg. Chem.* **1977**, *23*, 67. (b) Kepert, D. L. *Prog. Inorg. Chem.* **1979**, *25*, 41. (c) Kepert, D. L. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds.; Pergamon: Oxford, England, 1987; Vol. 1, p 31.

(28) (a) Palenik, G. J.; Wester, D. W. *Inorg. Chem.* **1978**, *17*, 864. (b) Lainé, P.; Gourdon, A.; Launey, J. P.; Tuchagues, J. P. *Inorg. Chem.* **1995**, *34*, 5150.

(29) Bencini, A.; Bianchi, A.; Dapporto, P.; Garcia-Espana, E.; Marcelino, V.; Micheloni, M.; Paoletti, P.; Paoli, P. *Inorg. Chem.* **1990**, *29*, 1716.

(30) (a) Nishida, Y.; Tanaka, N.; Yamazaki, A.; Tokii, T.; Hashimoto, N.; Ide, K.; Iwasama, K. *Inorg. Chem.* **1995**, *34*, 3616−3620. (b) Szulblinski, W. S.; Warburton, P. R.; Bush, D. H. *Inorg. Chem.* **1993**, *32*, 5368−5376. (c) Webley, W. S.; Durand, R. R., Jr.; Anson, F. C. *J. Electroanal. Chem.* **1987**, *229*, 273−283. (d) Costa, G.; Tavagnacco, C. *Gazz. Chim. Ital.* **1995**, *125*, 243−261.

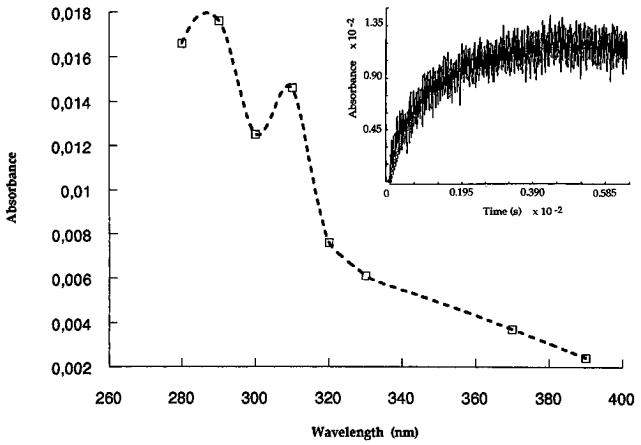


Figure 4. Difference absorption spectrum of the transient formed upon reaction of superoxide with Mn−TPAA. Superoxide free radicals were produced by pulse radiolysis of aqueous solution. Conditions: [Mn−TPAA] = 0.1 mM; formate, 0.1 M, pH 7.8; phosphate buffer, 1 mM; $P(O_2) = 1$ atm; dose 10 Gy; $[O_2^-] = 6.2$ μM; $t = 8$ ms after the pulse. Inset: kinetic trace at 320 nm.

indicates the existence of a reversible interaction of the complex with dioxygen. For a concentration of oxygen larger than that of the metal complex, the current intensity of the irreversible new wave increases with complex concentration.

All these observations support the interpretation of a complex-assisted reduction of oxygen and also indicate that the complex scavenges superoxide anion. In analogous examples, a precursor complex is generally deemed to be formed prior to reduction.[30] In similar studies performed on iron(II) complexes, a Fe(III)−peroxo adduct has been supposed to be formed as an intermediate in the reaction.[30b] Pulse radiolysis experiments shed complementary light on the fate of the system (*vide infra*).

Pulse Radiolysis. The reactivity of Mn−TPAA with superoxide was studied under conditions where $[Mn-TPAA]_0 < [O_2^-]_0$ ([Mn−TPAA] = 0.1 − 1.5 μM, $[O_2^-] = 6.2$ μM) whereas the chemical reaction of O_2^- with Mn−TPAA was investigated at $[Mn-TPAA]_0 \gg [O_2^-]_0$ ([Mn−TPAA] = 100 μM, $[O_2^-] = 6.2$ μM). If the complex catalyzes superoxide dismutation, the decay of the absorbance of superoxide would be faster and would follow pseudo-first-order kinetics. When $[Mn-TPAA]_0 < [O_2^-]_0$, we observed that the decay of superoxide, followed at 250 nm, is unperturbed and is analyzed to be the second-order self-dismutation rate obtained in the absence of complex. This result shows that Mn−TPAA does not efficiently catalyze the dismutation of superoxide. Then the reaction of O_2^- with Mn−TPAA was investigated. We have followed the absorption of the solution at different wavelengths, and we have observed that O_2^- reacts with Mn−TPAA, leading to a transient whose difference absorption spectrum, recorded 8 ms after the pulse and shown in Figure 4, is characterized by a peak at 310 nm. The inset of Figure 4 shows the kinetic trace of the transient formation at 320 nm. Kinetic analysis of such traces indicates that the rate constant of the reaction, k, is equal to $(1.05 \pm 0.1) \times 10^7$ M^{-1} s^{-1}. This rate is high and is of the same order of magnitude as those already determined for the reaction of manganese porphyrins[7] with superoxide. Preliminary experiments indicate that this transient decays with a half-life of *ca*. 0.2 s.

To obtain more information about the nature of the transient, we have quantified H_2O_2 formation upon reaction of O_2^- with Mn−TPAA. We have verified that the complex does not react with H_2O_2 in water. A steady state of superoxide radicals was produced by γ irradiation with a dose rate equal to 2 Gy min^{-1}. The subsequent formation of H_2O_2 was followed as a function

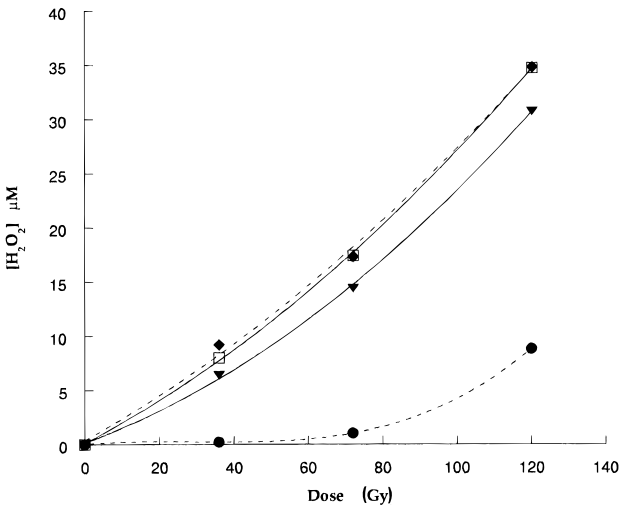


Figure 5. H_2O_2 production after reaction of various Mn−TPAA concentrations with superoxide. Superoxide free radicals were produced by γ irradiation of aqueous solutions. Conditions: formate, 0.1 M, pH 7.8; phosphate buffer, 1 mM; $P(O_2) = 1$ atm; dose rate 2 Gy min^{-1}. Key: □, blank, no complex added; ◆, [Mn−TPAA] = 5 μM; ▼, [Mn−TPAA] = 10 μM; ●, [Mn−TPAA] = 100 μM.

of the dose in the presence of different Mn−TPAA concentrations ([Mn−TPAA] = 0−100 μM) (Figure 5). Without the complex (blank), we find a yield of H_2O_2 in agreement with spontaneous O_2^- dismutation (3.6×10^{-7} mol J^{-1}). In the presence of Mn−TPAA (100 μM), H_2O_2 formation is inhibited, and the small quantity of H_2O_2 detected is only due to spur reactions in water radiolysis[31] ($G(H_2O_2) = 0.7 \times 10^{-7}$ mol J^{-1}). This inhibition is dependent on the Mn−TPAA concentration (Figure 5). This result shows that O_2^- does not disproportionate anymore and that the reaction of O_2^- with Mn−TPAA does not produce H_2O_2. The following reactions between Mn−TPAA and O_2^- can be symbolized:

$$LMn^{2+} = [Mn-TPAA]^{2+}$$

$$LMn^{2+} + O_2^- \rightarrow [LMn^{2+}-O_2^-]^+ \qquad k \approx 1 \times 10^7\ M^{-1}\ s^{-1} \quad (1)$$

$$[LMn^{2+}-O_2^-]^+ \rightarrow \text{product(s)} \quad (2)$$

It is known that the pK_a of $HO_2^\bullet/O_2^-$ is 4.7. Pulse radiolysis experiments are performed at pH 7.8, so that only reaction 1 occurs. The transient observed on reaction between Mn−TPAA and O_2^- is probably $[LMn^{2+}-O_2^-]^+$, (which is equivalent to $[LMn^{3+}-O_2^{2-}]^+$). The absence of H_2O_2 production suggests that reaction 3 does not occur. The transient should subse-

$$[LMn^{2+}-O_2^-]^+ \xrightarrow{2H^+} LMn^{3+} + H_2O_2 \quad (3)$$

quently disappear through reaction 2 in which the product is undetermined; we have no indication that a $[Mn-TPAA]^{3+}$ complex is formed. These results are in agreement with electrochemistry experiments which have shown that (i) the complex is very stable in the Mn(II) state and (ii) there is no evidence for $[Mn-TPAA]^{3+}$formation, even at high potential. Our observations are also in agreement with previously reported pulse radiolysis studies on Mn^{2+} free cations and Mn^{2+}−formate

(31) Buxton, G. V. In *Radiation Chemistry Principles and Applications*; Farhataziz, Rogers, M. A. J., Eds.; VCH: New York, 1987.

complexes. These two systems have been found to react with superoxide to form a $[MnO_2]^+$ transient which disappears without formation of a Mn(III) species.[32]

Conclusions

For the first time, a crystallographic structure containing the tripodal potentially heptadentate ligand TPAA chelating a metal has been completely characterized. The association of this ligand with manganese(II) forms a new seven-coordinate complex. The reaction of the complex with superoxide has been investigated. A clear demonstration is given that this molecule acts as a good stoichiometric, not catalytic, scavenger of superoxide despite the steric hindrance of the ligand. The first step of this reaction leads to an intermediate, $[(Mn-TPAA)^{2+}-O_2^-]^+$, detected by electrochemistry and by its difference absorption spectrum in pulse radiolysis. Work is in progress in order to elucidate the kinetic scheme of this reaction, especially the fate of hydrogen peroxide and the possible electron transfer mechanism.

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Supporting Information Available: Figure showing cyclic voltammograms for a fixed concentration of the complex and several dioxygen concentrations and tables of atomic coordinates, anisotropic thermal parameters, and bond lengths and angles (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(32) (a) Bielski, B. H. J.; Chan, P. C. *J. Am. Chem. Soc.* **1978**, *100*, 1920. (b) Bielski, B. H. J.; Cabelli, D. E. *J. Phys. Chem.* **1984**, *88*, 6291.