Inorganic Chemistry Cite This: Inorg. Chem. XXXX, XXX, XXX-XXX

Article pubs.acs.org/IC

Nitric Oxide Dioxygenase Activity of a Nitrosyl Complex of Mn(II)-Porphyrinate in the Presence of Superoxide: Formation of a Mn(IV)oxo Species through a Putative Peroxynitrite Intermediate

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ABSTRACT: A nitrosyl complex of Mn^{II}-porphyrinate, [(F₂₀TPP)- $Mn^{II}(NO)$], 1 (F₂₀TPPH₂ = 5,10,15,20 *tetrakis*(pentafluorophenyl)porphyrin), was synthesized and characterized. Spectroscopic and structural characterization revealed complex 1 as a penta-coordinated Mn^{II}-nitrosyl with a linear Mn-N-O (180.0°) moiety. Complex 1 does not react with O_2 . However, it reacts with superoxide (O_2^{-}) in THF at -80 °C to result in the corresponding nitrate (NO₃⁻) complex, 2, via the formation of a presumed Mn^{III}-peroxynitrite intermediate. ESI-mass spectrometry and UV-visible and X-band EPR spectroscopic studies suggest the generation of Mn^{IV}-oxo species in the reaction through homolytic cleavage of the O-O bond of the peroxynitrite ligand as proposed in NOD activity. The intermediate



formation of the Mn^{III}-peroxynitrite was further supported by the well accepted phenol ring nitration which resembles the biologically well-established tyrosine nitration.

INTRODUCTION

Nitric oxide (NO) is known to play important roles in mammalian biology including neurotransmission, immune response, etc.^{1,2} However, overproduction of NO has detrimental effects via the formation of secondary reactive nitrogen species (RNS), for example, nitrogen dioxide (NO_2) or peroxynitrite (ONOO⁻).^{3,4} The reaction of NO with a superoxide radical $(O_2^{\bullet-})$, hydrogen peroxide (H_2O_2) , and/or in the presence of transition metal ions is known to be responsible for the formation of these RNS.³ Nitric oxide dioxygenases (NODs) control the level of NO by converting it into nitrate (NO₃⁻).^{5,6} In NOD reactions, an iron(III)superoxo species reacts with NO to result in the biologically benign NO₃⁻ ion. The formation of a ONOO⁻ ion has been proposed as the intermediate in this transformation. It is proposed that an oxo-ferryl, [Fe^{IV}(O)] species formed in the reaction via the homolytic cleavage of the O-O bond of the Fe^{III}-(ONOO⁻) intermediate. Such an oxo-ferryl species has been evidenced spectroscopically in the reaction of myoglobin with the ONOO⁻ ion.⁷ Though, in the literature, the involvement of a proposed metal-peroxynitrite species was exemplified in the reactions of NO with metal-oxygen species, the direct evidence is still elusive. This is because of the very short lifetime of metal-peroxynitrite. In addition, the evidence of formation of high valent metal-oxo species from the homolytic O-O bond cleavage of the metal-peroxynitrite intermediate is also scarce because of very fast recombination of the metal-oxo species with NO2 in the reaction cage to result in NO₃⁻. Hence, the reactivity study of metal-superoxo species with NO or a metal-nitrosyl with superoxide is of immense importance to elucidate the undetected intermediate/s in the reaction.

In the literature, the reaction of superoxo and peroxo complexes of heme and nonheme iron, cobalt, and chromium with NO are reported where the formation of the metalperoxynitrite intermediates is implicated.⁸⁻¹⁰ For instance, in addition to the examples of formation of peroxynitrites in the reaction of oxy-heme (formally, $Fe^{III} - O_2^{-}$) proteins with NO, it has been reported recently that a Cr(IV)-peroxo complex $([Cr^{IV}-(12-TMC)(O_2)(Cl)]^+)$ {12-TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane} reacts with NO to lead to a Cr(III)-nitrato complex, ([Cr^{III}(12-TMC)(NO₃)-(Cl)⁺). On the other hand, the Cr(III)-superoxo analogue, $[Cr^{III}(14-TMC)(O_2)(Cl)]^+$, upon reaction with NO yielded the corresponding Cr(IV)-oxo complex, ([Cr^{IV}-(14-TMC)-(O)(Cl)⁺) and NO₂. In both the reactions, the involvement of a Cr(III)-peroxynitrite intermediate, [Cr^{III}(14-TMC)-(OON=O)(Cl)]⁺ was presumed.^{11,12,9d,e} The reaction of an Fe(III)-peroxo complex of the same ligand, [Fe^{III}(14-TMC)- (O_2)]⁺, with NO⁺ was also reported to proceed through the peroxynitrite intermediate.¹³ An example of the formation of a presumed peroxynitrite intermediate in the reaction of the cobalt-peroxo complex of a nonporphyrinate ligand with NO was reported recently from our laboratory.¹

On the other hand, presumed metal-peroxynitrites are reported to form in the reaction of a metal-nitrosyl and dioxygen (O_2) . For example, the reaction of cobalt-nitrosyl

Received: August 5, 2019

with O_2 to yield NO_2^- was reported long before.¹⁵ The reaction of a nonheme dinitrosyliron complex with O_2 resulted in a NO_3^- complex.¹⁶ A Cu(I)-nitrosyl complex afforded the NO_2^- product in the presence of O_2 via a presumed peroxynitrite intermediate.¹⁷ In addition, the {Cu(NO)}¹⁰ complex in the presence of H₂O₂ was also found to result in a copper-nitrato complex through a presumed Cu(I)-peroxynitrite intermediate formation.¹⁸

We report herein the synthesis and characterization of a five coordinated manganese-nitrosyl, $[(F_{20}TPP)Mn(NO)]$ (1), and its reactivity with superoxide (O_2^-) where the formation of peroxynitrite intermediate is implicated. Spectroscopic evidence suggests the formation of the $[(F_{20}TPP)Mn^{IV}(O)]$ species in the reaction. Finally, this species resulted in the corresponding NO_3^- product upon recombination with NO_2 .

EXPERIMENTAL SECTION

Materials and Methods. All reagents and solvents of reagent grade were purchased from commercial sources and used as received except as specified. All the reactions were performed under inert conditions unless specified. Deoxygenation of the distilled solvents and solutions was effected by repeated vacuum/purge cycles or bubbling with argon. UV-visible spectra were monitored on an Agilent Technologies Cary 8454 UV-visible spectrophotometer. FT-IR spectra of the samples were taken on a PerkinElmer spectrophotometer, and the samples were prepared either as KBr pellets or in solution in a KBr cell. A 400 MHz or 600 MHz Varian FT spectrometer was used for ¹H NMR spectra recording. Chemical shifts (ppm) were referenced either with an internal standard (Me₄Si) or to the residual solvent peaks. The X-band Electron Paramagnetic Resonance (EPR) spectra were recorded on a JES-FA200 ESR spectrometer, at room temperature as well as liquid nitrogen temperature with microwave power, 0.998 mW; microwave frequency, 9.14 GHz; and modulation amplitude, 2. Elemental analyses were obtained from a PerkinElmer Series II Analyzer.

Single crystals were grown by the slow evaporation of CHCl₃ and CH₂Cl₂ solutions of the ligand and complexes, respectively. The intensity data were collected using a Bruker SMART APEX-II CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube Mo K α radiation ($\lambda = 0.71073$ Å) at 293(3) K, with increasing ω (width of 0.3° per frame) at a scan speed of 3 s/frame. The SMART software was used for data acquisition. Data integration and reduction were undertaken with SAINT and XPREP software.¹⁹ Multiscan empirical absorption corrections were employed to the data using the program SADABS.²⁰ Structures were solved by direct methods using SHELXS-2016 and refined with full-matrix least-squares on F^2 using SHELXL-2016/6.²¹ Structural illustrations have been drawn with ORTEP-3 for Windows.²²

Syntheses. *Ligand* [(5,10,15,20-tetrakis(pentafluoropheny1)porphyrin)], ($F_{20}TPPH_2$). The ligand $F_{20}TPPH_2$ was prepared by mixing 2,3,4,5,6-pentafluorobenzaldehyde (3.92 g, 20 mmol) and freshly distilled pyrrole (1.34 g, 20 mmol) in 37 mL of propionic acid following the reported procedure of porphyrin synthesis.^{23a} The solution was refluxed for 3 h and cooled to room temperature. Neutralization of the reaction mixture was done by aqueous Na₂CO₃ to precipitate out the crude product. This crude product was purified by column chromatography as purple crystals (0.49 g, *ca.* 10% yield). Elemental analyses for C₄₄H₁₀F₂₀N₄, Calcd (%): C, 54.23; H, 1.03; N, 5.75. Found (%): C, 54.33; H, 1.07; N, 5.84. FT-IR (in KBr): 3318, 2923, 1648, 1514, 1500, 1435, 1078, 989, 919, 807, 772, 757, and 724 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ_{ppm} , 8.93 (s, 8H). Mass (m/Z) calcd: 974.06. Found: 975.03 (M+1).

Synthesis of $[Mn^{III}(F_{20}TPP)CI]$. The ligand $F_{20}TPPH_2$ (0.24 g, 0.25 mmol), $Mn(OAc)_2$ ·4H₂O (0.61 g, 2.50 mmol), and sodium chloride (0.09 g, 1.5 mmol) in CHCl₃(12 mL) and acetic acid (12 mL) was taken in a 100 mL round-bottom flask. The mixture was refluxed at 120 °C for 6 h. The solution was cooled and filtered. A total of 60 mL of water was poured into the filtrate. The solution was then extracted

with CHCl₃ (3 × 30 mL). The crude mass was column chromatographed to result in complex [Mn^{III}(F_{20} TPP)Cl] as a red solid.^{23b} Yield: 0.11 g (*ca.* 40%). Elemental analyses for C₄₄H₈N₄F₂₀ClMn, calcd (%): C, 49.72; H, 0.76; N, 5.27. Found (%): C, 49.80; H, 0.81; N, 5.39. UV–visible (THF): 429 nm (ε/M^{-1} cm⁻¹, 1.11 × 10⁵) and 561 nm (ε/M^{-1} cm⁻¹, 1.34 × 10⁴). FT-IR (in KBr): 1651, 1516, 1492, 1426, 1340, 1165, 1085, 1059, 1011, 989, 943, and 761 cm⁻¹. Mass (m/Z) calcd: 1026.98. Found: 1027.01 (molecular ion peak for [Mn^{III}(F_{20} TPP)]). Crystal data: [Mn^{III}(F_{20} TPP)Cl], CCDC No.1911995. C₄₄H₈F₂₀ClN₄Mn, M = 1062.93, tetragonal (I4/m), a = 17.639(3), b = 17.639(3), c = 9.583(2) Å, $\alpha = 90.00^{\circ}$, $\beta = 90.00^{\circ}$, $\gamma = 90.00^{\circ}$, V = 2981.6(12) Å³, Z = 2, Dc = 1.184 g cm⁻³, $\mu = 0.357$ mm⁻¹, T = 293(2) K, 1399 reflections, 570 independent, R(F) = 0.1247 [$I > 2\delta(I$]], R(int) = 0.1689, wR(F2) = 0.2537 (all data), GOF = 1.164.

Synthesis of Complex 1, $[Mn''(F_{20}TPP)(NO)]$. Complex 1 was prepared following the reported procedure with minor modification.^{23c} Triethylamine (0.76 g, 7.50 mmol) was added to a methanolic (10 mL) solution of hydroxylamine hydrochloride (0.52 g, 7.50 mmol) at 0 °C. A fresh solution of hydroxylamine was added to the CH_2Cl_2 solution of the $[Mn^{III}(F_{20}TPP)Cl]$ complex (1.59 g, 1.50 mmol) at -20 °C. Then, complex 1 precipitated out of solution as red crystals in a period of 3-4 days at low temperature and was isolated by filtration. Yield: 1.11 g, ~70%. Elemental analyses for C44H8N5OF20Mn, calcd (%): C, 49.98; H, 0.76; N, 6.62. Found (%): C, 50.06; H, 0.73; N, 6.70. UV-visible (THF): 415 nm (ε/M^{-1} cm⁻¹, 1.04 × 10⁵), 532 nm (ε/M^{-1} cm⁻¹, 1.02 × 10⁴), and 564 nm $(\varepsilon/M^{-1} \text{ cm}^{-1}, 3.95 \times 10^3)$. FT-IR (in KBr): 1763, 1650, 1516, 1489, 1344, 1195, 1165, 1079, 1059, 987, 941, and 762 cm⁻¹. ¹H NMR (400 MHz, d_6 -DMSO): δ_{ppm} 9.09 (s, 8H). Crystal data: complex 1, CCDC No. 1911994. $C_{48}H_{16}Cl_8F_{20}N_6O_2Mn$, M = 1427.21, tetragonal (I4/m), a = 16.8084(4), b = 16.8084(4), c = 9.5523(4) Å, $\alpha = 90.00^{\circ}$, $\beta = 90.00^{\circ}, \gamma = 90.00^{\circ}, V = 2698.74(17) \text{ Å}^3, Z = 2, Dc = 1.756 \text{ g cm}^{-3},$ $\mu = 0.757 \text{ mm}^{-1}$, T = 101(1) K, 1259 reflections, 1126 independent, $R(F) = 0.0477 [I > 2\delta(I)], R(int) = 0.0535, wR(F2) = 0.1292$ (all data), GOF = 1.032.

Synthesis of Complex 2, $[Mn^{ll}(F_{20}TPP)(NO_3)]$. Complex 1 (0.53 g, 0.5 mmol) was dissolved in 20 mL of dry THF, and the solution was cooled at -80 °C. To this cold solution, 1 equiv of potassium superoxide in the presence of 18-crown-6 (5 equiv) in THF was added at -80 °C and stirred for 2 h. The solution was slowly warmed to room temperature. The red solution changed to dark red. Then the solution was vacuum-dried (0.41 g, \sim 75% yield). Elemental analyses for C₄₄H₈N₅O₃F₂₀Mn, calcd (%): C, 48.51; H, 0.74; N, 6.43. Found (%): C, 48.59; H, 0.70; N, 6.56. UV–visible (THF): 458 nm (ε/M^{-1} cm⁻¹, 0.54 × 10⁵), 572 nm (ε/M^{-1} cm⁻¹, 0.77 × 10⁴). FT-IR (in KBr): 1650, 1525, 1492, 1384, 1335, 1162, 1073, 1052, 988, 937, 800, and 759 cm⁻¹. Mass (m/Z) calcd: 1088.97. Found: 1088.88 (molecular ion peak).

Complex **3**, $[Mn^{[II]}(F_{20}TPP)(OH)]$, 3,3',5,5'-tetra-tert-butyl-[1,1'biphenyl]-2,2'-diol and 2,4-ditertiarybutyl-6-nitrophenol. Complex **1** (1.06 g, 1 mmol) and 2,4-di-tert-butyl phenol (1.03 g, 5 mmol) were dissolved in 20 mL of dry and degassed THF. The mixture was cooled to -80 °C. To this degassed solution, 1 equiv of potassium superoxide in the presence of 18-crown-6 (5 equiv) in THF was added at -80 °C and stirred for 2 h. The reaction mixture was then brought to room temperature and dried under reduced pressure. The solid mass was then subjected to column chromatography using a silica gel column to obtain pure 3,3',5,5'-tetra-tert-butyl-[1,1'biphenyl]-2,2'-diol, 2,4-ditertiarybutyl-6-nitrophenol, and complex **3**.

¹3,3',5,5'-tetra-tert-butyl-[1,1'-biphenyl]-2,2'-diol. Yield: 0.08 g (~20%). Elemental analyses for C₂₈H₄₂O₂, calcd (%): C, 81.90; H, 10.31. Found (%): C, 81.98; H, 10.33. ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm ppm}$ 7.39 (s, 2 H), 7.12 (s, 2 H), 5.23 (s, 2 H), 1.45 (s, 18 H), and 1.32 (s, 18 H). ¹³C NMR (100 MHz, CDCl₃): $\delta_{\rm ppm}$ 150.0, 143.2, 136.5, 125.5, 125.0, 122.6, 35.4, 34.7, 31.9, and 29.9. Mass (m/Z) calcd: 410.32. Found: 409.31 (M – 1).

2,4-Di-tert-butyl-6-nitrophenol. Yield: 0.10 g (~40%), Elemental analyses for C₁₄H₂₁NO₃, calcd (%): C, 66.91; H, 8.42; N, 5.57. Found (%): C, 66.86; H, 8.45; N, 5.66. ¹H NMR (600 MHz, CDCl₃): δ_{ppm}

1.32 (s, 9H), 1.45 (s, 9H), 5.22 (s, 1H), 7.12 (s, 1H), 7.40 (s, 1H). ¹³C NMR (150 MHz, CDCl₃): $\delta_{\rm ppm}$ 29.7, 31.6, 34.5, 35.2, 122.3, 124.8, 125.3, 136.2, 143.0, 149.8. ESI-Mass (*m*/*z*) calcd: 251.15. Found: 250.41 (M - 1).

Complex 3. Yield: 0.68 g (~65%). Elemental analyses for $C_{44}H_9N_4OF_{20}Mn$, Calcd (%): C, 50.60; H, 0.87; N, 5.36. Found (%): C, 50.63; H, 0.89; N, 5.34. FT-IR (in KBr): 3530, 1650, 1490, 1423, 1163, 1087, 990, 761 cm⁻¹. UV–visible (THF): 430 nm (ϵ/M^{-1} cm⁻¹, 0.35 × 10⁵), 569 nm (ϵ/M^{-1} cm⁻¹, 0.49 × 10⁴).

RESULTS AND DISCUSSION

The ligand was prepared following typical reported methods of porphyrin synthesis and characterized using spectroscopic techniques (Supporting Information; Figures S1–S4).^{23a} The Mn^{II}-nitrosyl complex, {(F_{20} TPP)Mn(NO)} (1), has been prepared from the reaction of {(F_{20} TPP)Mn(Cl)} with hydroxylamine using the reported literature procedure with a little modification (Supporting Information; Figures S9–S12).^{23c} It was characterized by various spectroscopic techniques as well as by single crystal structure determination (Supporting Information; Tables S1–S3). The ORTEP diagram of complex 1 is given in Figure 1. It revealed complex



Figure 1. ORTEP diagram of complex **1** (50% thermal ellipsoid plot; H atoms and solvent molecules are removed for clarity).

1 as a five coordinated nitrosyl complex of Mn^{II} -porphyrinate. It is to be noted that though there are many examples of structurally characterized six coordinated nitrosyl complexes of Mn^{II} -porphyrinates, the number for five coordinated ones is much less. The metric parameters (Supporting Information; Tables S1–S3) are within the range of reported analogous complexes. The N–O bond distance and Mn–N–O bond angles are 1.132(10) Å and 180.0°, respectively. These values appear in the reported range for analogous complexes. For instance, in the case of the nitrosyl complex of (5,10,15,20tetratolylporphinato)manganese(II), the Mn–N–O bond angle is reported as 177.8(3)° and the N–O distance is 1.160(3) Å.²⁴

The complex 1 in THF solution at -80 °C shows absorption bands at 415, 532, and 564 nm (Figure 2 and Supporting Information, Figure S10). The addition of a precooled THF solution of KO₂ in crown ether resulted in the decrease of the 415 nm band along with a concomitant formation of new absorption bands at 426 and 458 nm (Figure 2; Scheme 1).



Figure 2. UV–visible spectral monitoring of complex 1 (black) and after addition of KO_2 in crown ether to result in complex 2 (red) in THF at -80 °C.

The 426 nm band was transient and disappeared with time, whereas the intensity of the absorption at 458 nm increases. The final decomposition product was isolated and characterized as the corresponding nitrate (NO₃⁻) complex of Mn^{III}porphyrinate complex 2. It shows an absorption band at 458 nm. The transient 426 nm band is attributed to the absorption of the corresponding Mn^{IV}-oxo species, which is formed via the homolytic O-O bond cleavage of a presumed Mn^{III}peroxynitrite intermediate (Scheme 1). In the literature, it has been reported that Mn^{IV}-oxo complexes absorb at the range of 420–430 nm and owing to their very unstable nature, convert rapidly to the corresponding Mn^{III}-porphyrinate. Newcomb et al. reported the formation of the same $[(F_{20}TPP)Mn^{IV}O]$ species in acetonitrile solution through laser flash photolysis of [(F20TPP)Mn^{III}(ClO3)], and that shows absorption at 422 nm.²

The minor shift in absorption wavelength is perhaps because of the change of solvent and temperature.

It is to be noted that when $[(F_{20}TPP)Mn^{II}]$ was made to react with KO₂ in THF at -80 °C, the absorption bands in the UV-visible spectrum appeared at 451 and 569 nm, respectively (Supporting Information, Figure S30), and these bands are stable at that temperature for hours. In the reaction of the $[(F_{20}TPP)Mn(NO)]$ and KO₂ in THF at -80 °C, we have not observed any such bands in the UV-visible monitoring. Hence, the possibility of the replacement of the NO group by superoxide could be ruled out in the reaction of $[(F_{20}TPP)Mn(NO)]$ and KO₂.

In FT-IR spectroscopy, complex 1 in a KBr pellet displays nitrosyl stretching at 1763 cm⁻¹ (Figure 3). This is in agreement with the reported nitrosyl stretching in analogous complexes. For example, [(TPP)Mn^{II}NO] shows nitrosyl stretching at 1760 cm^{-1.26} We attempted to monitor the FT-IR spectral change during the reaction; however, we ended up with a spectrum where the 1763 cm⁻¹ band disappeared with the appearance of a new stretching at ~1430 and 1261 cm⁻¹. These are assignable to the corresponding NO₃⁻ stretchings of complex 2 (Supporting Information, Figure S13b).²⁷ When the FT-IR spectrum of the isolated complex 2 was recorded in KBr pellets, a stretching at 1384 cm⁻¹ was observed (Supporting Information, Figure S13a). It is to be noted that in the case of [(TPP)Mn(ONO₂)], the nitrate stretching appeared at ~1470

Scheme 1





Figure 3. FT-IR spectrum of complex 1 in KBr.

and 1284 cm⁻¹. However, when the spectrum was recorded in KBr pellets, three bands were observed at 1474, 1385, and 1286 cm⁻¹. The appearance of the 1384 cm⁻¹ band was attributed to the stretching of free NO₃⁻, which is formed in the reaction of $[(TPP)Mn(O-NO_2)]$ with KBr.²⁷ In comparison with the other reported analogous complexes, the bands at 1430 and 1261 cm⁻¹ are assigned to the $\nu_a(NO_2)$ and $\nu_s(NO_2)$, respectively, of the uncoordinated NO₂ group in the $[Mn(^1\eta\text{-}ONO_2)]$ moiety, suggesting the presence of a nitrate ion in O-coordinated monodentate fashion.²⁷ The stretching corresponding to the coordinated O–N fragment appears in the range of ~1000 cm⁻¹. However, we have not observed that in the present case perhaps because of masking by the presence of strong porphyrin stretching bands.²⁷

The ESI mass spectrum of complex 1 was populated by the molecular ion peak at m/z 1027.15 assignable to the $[(F_{20}TPP)Mn]$ moiety (calcd. m/z = 1026.98; Supporting Information, Figure S11). This is attributed to the loss of an axial nitrosyl group under the experimental conditions.²⁸ However, the ESI-mass spectrum of the reaction mixture obtained from the reaction of complex 1 with KO₂ in THF at -80 °C shows the molecular ion peak at 1043.07, which is

assignable to the $[(F_{20}TPP)Mn(O)]$ moiety (calcd.1042.98; Figure 4).



Figure 4. ESI-mass spectrum of the reaction mixture obtained from the reaction of complex 1 and KO_2 (in crown ether) in THF at -80 °C. [Inset: (a) simulated and (b) experimental isotopic distribution pattern.]

The isotopic distribution pattern in the observed spectrum matches well with the simulated one (Figure 4). Thus, the ESI mass spectrometry suggests the formation of a $[(F_{20}TPP)Mn-(O)]$ intermediate. In the mass spectrum of the decomposition product, the molecular ion peak appeared at m/z 1088.88 corresponding to a $[(F_{20}TPP)Mn(NO_3)]$ unit (calcd m/z, 1088.97; complex 2; Supporting Information, Figure S14).

In X-band EPR spectroscopy, complex 1 appeared silent as expected for a low spin Mn^{II} center with an antiferromagnetically coupled nitrosyl group. However, the frozen (at 77 K) reaction mixture obtained from the reaction of complex 1 in THF at -80 °C with KO₂ displayed a strong, broad EPR signal with $g \sim 5.26$ and a weak one at 2.27, respectively. This is attributed to the presence of a high spin Mn^{IV} center (S = 3/2)

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in a square pyramidal geometry (Figure 5).²⁹ A spin quantification experiment with respect to the authentic



Figure 5. X-band EPR spectrum of the intermediate formed during the reaction between complex 1 and KO_2 (in crown ether) in THF at 77 K.

 $[Mn^{IV}=O]$ sample generated in the reaction of the $[(F_{20}TPP)MnCl]$ complex and *m*CPBA suggests the presence of ~60% of the same species in the frozen (at 77 K) sample obtained from the reaction of complex 1 and KO₂ in THF at -80 °C (Supporting Information, Figure S32a,b). [(TMP)-Mn^{IV}(O)] was reported to display a strong and broad signal at $g \sim 4$ and a weak one at $g \sim 2.21$.²⁹ These g values, however, are in the range of the reported Mn^{IV}=O complexes. For instance, $g_{\text{eff}} \sim 5.76$ signal in the case of $[Mn^{IV}(O)(N_4py)]^{2+}$ $[N_4py = (N,N-bis(2-pyridylmethyl)-N-bis(2-pyridyl)-methylamine)].³⁰$

In the case of $[Mn^{IV}(H_3 \text{ buea})(O)]$ species $[H_3\text{ buea}^{3-} = \text{tris}[(N'-tert-butylureaylato)-N-ethylene]aminato)], the g values appeared at 5.15, 2.44, and 1.63.³¹ To confirm the presence of <math>Mn^{IV}$ center, an authentic sample was prepared from the reaction of $[(F_{20}\text{ TPP})\text{MnCl}]$ with *m*CPBA in THF at -80 °C, and the EPR spectrum was recorded at 77 K and compared with the observed one (Supporting Information, Figure S24). It was found that both the spectra are in agreement. It should be worth mentioning that Mn(II) high spin (S = 3/2) complexes show signals at $g \sim 5.95$ and 2.10 in X-band EPR spectrum.³² To confirm our assignment of the observed signals, the EPR of the authentic $[(F_{20}\text{ TPP})\text{Mn}^{II}]$ was recorded in THF at 77 K (Supporting Information, Figures S25, S26). The respective signals appeared at $g \sim 6.10$ and 2.15.

Thus, UV–visible, ESI-mass, and X-band EPR spectral studies suggest the formation of a Mn^{IV} -oxo intermediate in the reaction of complex 1 with KO₂. We have tried to record the resonance Raman spectrum of the intermediate but could not succeed. This is perhaps because of thermal instability of the intermediate along with its photodecomposition under laser light. A probable mechanism of formation of the Mn^{IV} -oxo species in the reaction of Mn^{II} -nitrosyl complex 1 with O_2^- is depicted in Scheme 1. According to the widely accepted mechanism of NOD activity, the Fe^{III}-(O_2^-) species reacts with NO to result in the Fe^{III}-peroxynitrite intermediate. In the subsequent step, the peroxynitrite ligand undergoes homolytic O–O bond cleavage to generate Fe^{IV}-oxo species and NO₂.⁷ In the present study, thus the reaction of Mn^{II} -nitrosyl with O_2^- is presumably resulting in the corresponding Mn^{II} -peroxynitrite

intermediate, which gives rise to the Mn^{IV} -oxo and NO_2 following the NOD pathway.

The isolation and characterization of complex 2 as the decomposition product from the reaction of complex 1 and KO₂ also supports the formation of a Mn^{III}-peroxynitrite intermediate.^{27,28a} It is well established that the very short lifetime of the metal-peroxynitrite complexes precluded their spectral analyses. Therefore, the chemical evidence was sought to establish the intermediate formation of peroxynitrite in said reaction. When the reaction was carried out in the presence of 2,4-ditertiarybutylphenol, nitration was observed leading to 2,4-ditertiarybutyl-6-nitrophenol formation in ca. 40% yield with a fraction of oxidative product bis-phenol (ca. 20%). It is to be noted that phenol nitration/oxidative coupling has been used extensively as evidence to establish the presence of metalperoxynitrite species. This reaction has considerable importance as this resembles the biologically well-known tyrosine nitration.^{3a,b,4,33} In addition, complex 1 itself does not react with 2,4-ditertiarybutylphenol under the reaction conditions.

In the NOD reactivity of heme-iron complexes, it is proposed that metal-peroxynitrite ion mediated nitration or oxidation proceeds through the formation of the Fe^{IV}-oxo intermediate along with NO2. The phenolic substrates reduce the Fe^{IV}-oxo species rapidly to the Fe^{III} state to result in a phenoxyl radical. This radical then either combines with NO₂ to result in nitro-phenol or two phenoxyl radicals couple to yield corresponding bis-phenol product. In the present case, the spectral evidence suggests the formation of Mn^{IV}-oxo species in the reaction of complex 1 with KO₂. Recently, it has been reported that a Cr^{III}-superoxo complex of tetramethylated cyclam ligand reacts with NO in acetonitrile at -40 °C to result in the formation of Cr^{IV}-oxo species via homolytic O-O bond cleavage of the presumed Cr-peroxynitrite intermediate.^{9d} Earlier, cobalt nitrosyl $[(F_8TPP)Co(NO)]$ was found to show NOD reactivity in the presence of H₂O₂ through the formation of a transient $[(F_8TPP)Co^{III}-O^{\bullet}]$ radical.²⁷ Complexes of Cu and Cr were also reported in the literature to display NOD activity. However, to the best of our knowledge, this result demonstrates the first example of a nitrosyl complex of Mn-porphyrin, which displays NOD reactivity in the presence of KO₂. The spectral evidence clearly suggests the involvement of a Mn^{IV}-oxo intermediate in the NOD reactivity of complex 1.

CONCLUSIONS

Thus, the nitrosyl complex of Mn^{II} -porphyrinate, $[(F_{20}TPP)-Mn^{II}(NO)]$, **1**, upon reaction with superoxide (O_2^-) in THF at -80 °C resulted in the corresponding nitrate (NO_3^-) complex **2** via the formation of a presumed Mn^{III} -peroxynitrite intermediate. ESI-mass spectrometry, UV–visible, and X-band EPR spectroscopic studies suggest the generation of Mn^{IV} -oxo species in the reaction through homolytic cleavage of the O–O bond of the peroxynitrite ligand as proposed in NOD activity. The intermediate formation of the Mn^{III} -peroxynitrite was further supported by the well accepted phenol ring nitration, which resembles the biologically well-established tyrosine nitration.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorg-chem.9b02359.

Figures S1–S32 and Tables S1–S3 (PDF)

Accession Codes

CCDC 1911994–1911995 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Science and Technology, India for financial support (EMR/2014/ 000291) and DST-FIST for the single crystal X-ray diffraction facility. B.M., D.B., and R.M. extend their sincere thanks to the CIF, IIT Guwahati for instrumental facilities.

DEDICATION

Dedicated to Prof. G. K. Lahiri on his 60th birthday.

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