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Design and reactivity of Ni-complexes using pentadentate neutral-polypyridyl ligands: Possible mimics of NiSOD

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Abstract

Superoxide plays a key role in cell signaling, but can be cytotoxic within cells unless well regulated by enzymes known as superoxide dismutases (SOD). Nickel superoxide dismutase (NiSOD) catalyzes the disproportion of the harmful superoxide radical into hydrogen peroxide and dioxygen. NiSOD has a unique active site structure that plays an important role in tuning the potential of the nickel center to function as an effective catalyst for superoxide dismutation with diffusion controlled rates. The synthesis of structural and functional analogues of NiSOD provides a route to better understand the role of the nickel active site in superoxide dismutation. In this work, the synthesis of a series of nickel complexes supported by nitrogen rich pentadentate ligands is reported. The complexes have been characterized through absorption spectroscopy, mass spectrometry, and elemental analysis. X-ray absorption spectroscopy was employed to establish the oxidation state and the coordination geometry around the metal center. The reactivity of these complexes towards KO_2 was evaluated to elucidate the role of the coordination sphere in controlling superoxide dismutation reactivity.

Graphical abstract

Three nickel(II) complexes supported by pentadentate nitrogen rich ligands were probed for superoxide dismutation capabilities. Complexes were characterized through various techniques including X-ray absorption spectroscopy and absorption spectroscopy. All three complexes demonstrated superoxide reduction capabilities.

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Notes

The authors declare no competing financial interest

Supporting Information. Tables with EXAFS fits, an alternative visual presentation of the XANES spectra for 1–3, FcPF₆ titrations, NaI assay, and some reactivity data for complex 2.

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Keywords

ROS; nickel superoxide dismutase; synthesis; oxidation reactions

INTRODUCTION

Superoxide, a byproduct of aerobic metabolism, is a cellular toxin belonging to the class of reactive oxygen species (ROS).^{1,2} ROS are produced as a part of cellular metabolic processes. An excessive amount of ROS has been implicated in chronic inflammatory conditions, malignant neoplasms and various neurodegenerative diseases.^{3–7}

Metalloenzymes known as superoxide dismutases (SODs) are well documented as a cellular defense mechanism against superoxide.⁸ Specifically, SODs catalyze the dismutation (disproportionation) of superoxide ($\text{O}_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Three classes of SOD enzymes have been characterized to date, differing by the metal center at the active site: CuZn superoxide dismutase (CuZnSOD), the structurally related Fe Superoxide dismutase (FeSOD) and Mn superoxide dismutase (MnSOD), and Nickel superoxide dismutase (NiSOD).^{2,8,9} NiSOD, the most recently discovered member of this family, was located in soil and aquatic bacteria like *Streptomyces*¹⁰ and cyanobacteria,¹¹ and has a unique active site compared to other SOD enzymes.^{12,13} The nickel center in NiSOD is coordinated in a mixed nitrogen/sulfur coordination sphere (Figure 1), and cycles through Ni(II) and Ni(III) redox states throughout the catalytic process.¹³ In the reduced form, the low spin Ni(II) ion is four-coordinate and lies in a four-coordinate planar environment. However, in the oxidized form Ni(III) is five coordinate in square pyramidal geometry, with the fifth coordination site occupied by a neighboring histidine. Work with wild type NiSOD and mutated NiSOD proteins suggest that the axial histidine that binds to

the Ni(III) center is absolutely vital for superoxide dismutation.^{14–16} In the physiological pH range aquated Ni(II) does not react with superoxide, indicating that the protein derived ligands in NiSOD are responsible for lowering the Ni(II/III) redox potential to be within the window necessary for superoxide dismutation.^{15,17}

Although we have a clear understanding of the structural aspects of NiSOD due to detailed spectroscopic studies supported by computational calculations,¹⁸ a thorough knowledge of the mechanism of the enzyme remains elusive.¹⁹ NiSOD has an unusual anionic amide and free amine providing ligating nitrogen centers, and their relevance for controlling reactivity is not fully understood.²⁰ Biological reduction of superoxide to hydrogen peroxide has to be proton coupled, through one of the two possible mechanisms: a.) an inner sphere mechanism where the superoxide coordinates to the metal center and subsequently gets reduced to peroxide (or oxidized to oxygen); b.) via an outer sphere mechanism.¹ For NiSOD, the mechanism for SOD catalysis and whether an inner sphere pathway or outer sphere pathway is used remains under debate.^{2,21–24} An inner sphere mechanism will require a vacant site at the metal center, as well as clear tunnels for small molecule ingress/egress. However, in NiSOD, while the Ni site is located fairly close to the protein surface, access to the active site is blocked by a hook motif, making the Ni(II) center quite inaccessible for coordination of solvent or superoxide.² Thus, in the native enzyme, it is proposed that the reaction proceeds through an outer-sphere mechanism.^{18,23,25} In order to gain a fundamental understanding of the enzyme mechanism, many groups have employed different techniques to model NiSOD, with peptide maquettes proving to be especially useful.^{12,26} NiSOD biomimics using small peptide maquettes answered many important questions regarding the enzyme mechanism, and gave credence to a proton coupled electron transfer mechanism, where the thiolate ligand was protonated and acts as a source for the superoxide to peroxide transformation.^{27,28} However, designing a small, synthetic analogue without the peptide backbone remains a challenge. NiN₂S₂ and NiN₃S₂ complexes are either non-reactive or favor sulfur oxidation over metal mediated superoxide disproportionation when exposed to superoxides.^{13,29} In some cases, it has proven possible to electrochemically generate the Ni(III) oxidation state and spectroscopically characterize that fleeting intermediate.^{13,28,30–33} In our knowledge there are very few examples of low-molecular weight functional analogues of NiSOD that replicate reactivity. Almost all of them appeared in the last decade and they incorporate either one thiolate or thioether in the ligand framework.^{34–36} According to one such study providing a detailed mechanistic analysis of superoxide disproportionation by the synthetic analogues for NiSOD, superoxide reduction occurs via an inner-sphere mechanism and superoxide oxidation proceeds through an outer-sphere pathway.^{32,36} The first five-coordinated analogue of the NiSOD active site supported by an N₃O₂ ligand set was recently reported, and was shown to be the first example of a functional mimic of NiSOD.³⁷ Notably, the pentadentate ligand used did not have any sulfur atoms in the ligand framework, was able to stabilize the Ni(III) oxidation state, and performed superoxide oxidation.³⁷ However, the nickel complex with the analogous N₃S₂ ligand showed no SOD activity, although a Ni(III) species were obtained as a metastable intermediate.²⁹ To our knowledge most NiSOD mimics reported to date have been supported by anionic ligands, retaining the characteristic donor environment of the enzyme active site.^{20,32,35,38}

A focus in our group is to develop pentadentate Ni(II) complexes having neutral amine/pyridine ligand functionalities, and test their potential toward superoxide reactivity. There is documented literature evidence for nickel superoxo/peroxo intermediates stabilized by using neutral amine ligands, but their potential toward superoxide disproportionation has not been investigated.^{39–41} In this work, the synthesis, characterization, and reactivity of a family of Ni complexes supported by N5 ligands (Figure 2) towards superoxide is described. As previously stated, axial coordination by histidine in native NiSOD is key to stabilizing the Ni(III) oxidation state required for superoxide reduction.³² The pentadentate nature of the ligands in this study may facilitate stabilization of the Ni(III) redox state in a manner analogous to the enzyme, and can potentially replicate both the four coordinate planar and square pyramidal geometries seen in the native enzyme through on/off coordination of the pendant arm. Furthermore, it has been shown that modifying the steric bulk of the ligand can tune the redox potential of the metal center, and even direct the binding geometry of O₂ adducts.^{42–44} Therefore, we also sought to evaluate steric effects in the α -position in the pyridine ring by synthesizing the quinoline variation of the pentadentate ligand. Through a combination of geometric constraints imposed by ligand design and steric considerations, we hope to poise the redox potential of the nickel to support O₂^{•−} dismutation.

EXPERIMENTAL METHODS

Materials

The reagents and the solvents used in this study, except for the ligands and complexes, were commercial products of the highest available purity obtained from Sigma-Aldrich and Fisher Scientific. ¹H NMR experiments were performed in deuterated solvents purchased from Cambridge Isotope Laboratory on a Varian Inova-500 MHz instrument at 25 °C. UV-Visible experiments were performed on an Agilent Cary 8454 UV-Vis spectrometer in a quartz cuvette with a 1 cm pathlength. Electrochemical measurements were carried out on a BASi-C3 Epsilon electrochemical instrument using a glassy carbon working electrode, a platinum wire auxiliary electrode, and Ag/AgCl reference electrode. All cyclic voltammetric experiments were performed under nitrogen atmosphere. Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on a Thermo Scientific LTQ Orbitrap XL Hybrid FT Mass Spectrometer in positive ionization mode. Solid state magnetic susceptibility measurements were performed at room temperature using a MSB Evan's balance (model number MK I #7967; Johnson Matthey; Wayne, PA). The appropriate diamagnetic corrections were applied using Pascal's constants.⁴⁵ Elemental analyses were performed by Atlantic Microlab, Inc (Norcross, GA). N,N,N'-Tris(2-pyridylmethyl)-N'-Methylethylenediamine (TPMEN) and N'-phenyl-N,N,N'-tris(2-pyridylmethyl)-1,2-Ethanediamine (PhTPEN) were synthesized according to reported procedures.^{46,47} **Caution!** Although no problems were encountered in the preparation of the perchlorate salts, care should be taken when handling such potentially hazardous compounds.

Synthesis of N,N,N'-Tris(2-pyridylmethyl)-N'-Methylethylenediamine (TPMEN)

—2-Picolyl chloride hydrochloride (3.0 g, 18.3 mmol) was dissolved in 10 N NaOH (30 mL) aqueous solution. To this solution N-Methylethylenediamine (0.45 mL, 5.2 mmol) was added dropwise with stirring. After stirring for 3 days at room temperature, the upper oil

layer was decanted and dissolved in dichloromethane (30 mL). The dichloromethane solution was washed with an excess of water (80 mL) and dried using anhydrous MgSO_4 . After evaporation of dichloromethane, the crude sample was purified using a silica column ($\text{CHCl}_3:\text{CH}_3\text{OH}$ 10:1 (v/v)). A light brownish oil was obtained with a 50% yield (0.90 g, 2.6 mmol). NMR δ (500 MHz, CDCl_3) 8.51 (m, 3H, -Py), 7.62 (m, 3H, -Py), 7.52, 7.37 (m, 3H, -Py), 7.13 (m, 3H, -Py), 3.85 (s, 4H, $-\text{N}(-\text{CH}_2\text{Py})_2$), 3.48 (s, 2H, $-\text{N}-\text{CH}_2\text{Py}$), 2.77 (d, 4H, $-\text{N}-\text{CH}_2\text{CH}_2\text{N}$), 2.20 (s, 3H, $-\text{CH}_3$).

Synthesis of N'-phenyl-N,N,N'-Tris(2-pyridinylmethyl)-1,2-Ethanediamine (PhTPEN)—2-Picolyl chloride hydrochloride (3.0 g, 18.30 mmol) was dissolved in 10 N

NaOH (25 mL) aqueous solution. To this solution, N-phenylethylenediamine (0.71 g, 0.68 mL, 5.20 mmol) was added dropwise with stirring. After 3 days stirring at room temperature, the upper oil layer was decanted and dissolved in dichloromethane (30 mL). The dichloromethane solution was washed with an excess of water (80 mL) and dried using anhydrous MgSO_4 . After evaporation of dichloromethane, the crude sample was purified using a silica column ($\text{CHCl}_3:\text{CH}_3\text{OH}$ 10:1 (v/v)). A light yellowish oil was obtained with a 75% yield (1.6 g, 3.9 mmol). NMR δ (500 MHz, CDCl_3) 8.52 (m, 3H, -Py), 7.62 (m, 3H, -Py), 7.60 (m, 3H, -Py), 7.14 (m, 3H, -Py), 6.52 (m, 4H, -Ph), 4.57 (s, 2H, $-\text{N}-\text{CH}_2\text{Py}$), 3.90 (s, 4H, $-\text{N}(-\text{CH}_2\text{Py})_2$), 3.63-2.58 (d, 4 H, $-\text{N}-\text{CH}_2\text{CH}_2\text{N}$).

Synthesis of N,N,N'-Tris(2-quinolinemethyl)-N'-Methylethylenediamine (TQMEN)—2-(chloromethyl) quinoline hydrochloride (3.0 g, 14.01 mmol) was dissolved in

10 N NaOH (30 mL) aqueous solution. To this solution, N-Methylethylenediamine (0.347 mL, 3.98 mmol) was added dropwise with stirring. After 3 days at room temperature, the upper oil layer was decanted and dissolved in dichloromethane (30 mL). The dichloromethane solution was washed with an excess of water (80 mL) and dried using anhydrous MgSO_4 . After evaporation of dichloromethane, the crude sample was purified using a silica column ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ 10:1 (v/v)). Light yellowish oil of the ligand was obtained with 67% yield (1.3 g, 2.7 mmol). ^1H NMR δ (500 MHz, CDCl_3) 8.09 (m, 3H, -Qn), 8.01 (m, 3H, -Qn), 7.86 (m, 3H, -Qn), 7.80 (m, 3H, -Qn), 7.65 (m, 3H, -Qn), 7.55 (m, 3H, -Qn), 5.65-5.36 (d, 4H, $-\text{N}-\text{CH}_2\text{CH}_2\text{N}$), 4.28 (s, 3H, $-\text{CH}_3$), 3.48 (s, 4H, $-\text{N}(-\text{CH}_2\text{Qn})_2$), 3.40 (s, 2H, $-\text{N}-\text{CH}_2\text{Qn}$). ^{13}C NMR (500 MHz, CDCl_3) 158.68, 150.41, 147.45, 137.57, 136.76, 130.15, 129.67, 129.20, 128.96, 127.83, 127.70, 127.62, 127.22, 126.44, 125.03, 121.23, 65.56, 61.28, 57.84, 48.93.

Synthesis of $[\text{Ni}(\text{TPMEN})(\text{ClO}_4)_2]$ (1)—A solution of $[\text{Ni}(\text{ClO}_4)_2]\cdot 6\text{H}_2\text{O}$ (0.366 g, 1 mmol) in methanol was added to a solution of TPMEN (0.347 g, 1 mmol) also in methanol. The dark red solution was stirred at room temperature for 30 min. After 30 minutes acetonitrile (5 mL) was added to the solution and stirred for an additional 30 mins. The solution was then dried *in vacuo* before washing with diethyl ether (2 mL), and again drying *in vacuo* at 40 °C. A mauve colored solid was obtained in 64% yield (0.387 g, 0.64 mmol) (ESI-MS m/z for $[\text{Ni}(\text{TPMEN})(\text{ClO}_4)]^+$: 504.10 (calculated), 504.25 (experimental). Elemental analysis calculated for $[\text{Ni}(\text{TPMEN})(\text{ClO}_4)_2]\cdot 2\text{H}_2\text{O}$: C, 39.34; H, 4.56; N, 10.92; Cl, 11.06%. Found: C, 39.15; H, 4.44; N, 10.82; Cl, 11.45%. UV-Vis (λ (nm) [ϵ ($\text{M}^{-1}\text{cm}^{-1}$)] in MeOH at 25 °C: 545 (26), 908 (30).

Synthesis of [Ni(PhTPEN)(ClO₄)₂] (2)—A solution of [Ni(ClO₄)₂]•6H₂O (0.180 g, 0.492 mmol) in methanol was added to a solution of PhTPEN (0.202 g, 0.493 mmol) also in methanol. The dark purple solution was stirred at room temperature for 45 min. After 45 minutes of stirring acetonitrile (5 mL) was added to the solution and stirred for an additional 30 minutes. The solution was then dried *in vacuo* to obtain a lilac solid (0.223 g, 0.33 mmol, yield: 68%). ESI-MS *m/z* for [Ni(PhTPEN)(ClO₄)]⁺: 566.11(theoretical), 566.25 (experimental). Elemental analysis calculated for [Ni(PhTPEN)(ClO₄)₂] • MeCN: C, 47.49; H, 4.27; N, 11.87; Cl, 10.01%. Found: C, 47.32; H, 4.26; N, 11.85; Cl, 9.75%. UV-Vis (λ (nm) [ε (M⁻¹cm⁻¹)] in MeOH at 25 °C: 560 (19), 1040 (22).

Synthesis of [Ni(TQMEN)(ClO₄)₂] (3)—A solution of [Ni(ClO₄)₂]•6H₂O (0.182 g, 0.498 mmol) in methanol was added to a solution of TQMEN (0.248 g, 0.498 mmol) also in methanol. The green solution was stirred at room temperature for 30 min. The solution was then dried *in vacuo* to obtain a green solid (0.304 g, 0.40 mmol, yield: 81%). ESI-MS *m/z* for [Ni(TQMEN)(ClO₄)]⁺: 654.14 (calculated), 654.25 (experimental). Elemental analysis calculated for [Ni(TQMEN)(ClO₄)₂]•2H₂O•0.5HCl: C, 48.98; H, 4.42; N, 8.65; Cl, 10.95%. Found: C, 48.83; H, 4.77; N, 8.00; Cl, 10.58%. UV-Vis (λ (nm) [ε (M⁻¹cm⁻¹)] in MeOH at 25 °C: 411 (56), 685 (10).

XAS Data Collection & Analysis

X-ray absorption spectroscopy data was collected at beamline 2-2 of the Stanford Synchrotron Radiation Lightsource (SLAC National Accelerator Lab, Menlo Park, CA), with the storage ring operating at 3.0 GeV and 500 mA. A Si(111) double crystal monochromator was used for energy selection, and was detuned by ~30% for higher harmonic rejection. Samples were kept at ~20 K during data collection with a He Displex cryostat. A nickel metal foil spectrum was recorded simultaneously using a photodiode for internal energy calibration, with the first inflection point of the reference foil edge set to 8333.0 eV. XAS data were collected as fluorescence spectra using a 13 element solid state germanium detector (Canberra), with the following parameters: 10 eV steps/1 second integration time in the pre-edge region, 0.3 eV steps/2 second integration time in the edge region, and 0.05 *k* steps in the EXAFS region, with integration times increasing in a *k*²-weighted fashion from 2 to 9 seconds over the energy range of the scan (*k*_{max} = 15 *k*). The total detector counts were typically 25–35 kHz, well within the linear range of the analog detector electronics. Samples were monitored for photoreduction during data collection. No photoreduction was observed for any sample based on the absence of any scan-to-scan red-shift in the absorption edge. Tandem Mossbauer/XAS cups with sample windows of 3 mm × 10 mm were used as sample cells.

Averaging and normalization of the XAS data was performed using Athena.⁴⁸ EXAFS analysis was carried out using Artemis, which incorporates the IFEFFIT fitting engine and FEFF6 for *ab initio* EXAFS phase and amplitude parameters.⁴⁸ Appropriate crystal structures were used for FEFF6 input to identify significant paths. For a given shell, the coordination number *n* was fixed, while *r* and σ² were allowed to float. The amplitude reduction factor *S*₀² was fixed at 0.9, while the edge shift parameter *E*₀ was allowed to float at a single common value for all shells. For simulations incorporating multiple

scattering paths, σ^2 for the multiple scattering path was floated at a common value with its corresponding single-scattering path, while r was allowed to freely float. The fit was evaluated in k^3 -weighted R-space, and fit quality was judged by the reported R-factor and reduced χ^2 . Significant fits are tabulated in Tables S1–S3.

Oxidation with Ferrocenium hexafluorophosphate (FcPF₆)

The complexes **1–3** were dissolved in DMSO and kept under N₂. Similarly, FcPF₆ was dissolved in DMSO and stored under N₂ to avoid air oxidation. The complexes were titrated with FcPF₆ by adding aliquots of 0.2 equivalents FcPF₆ at a time. Titration occurred under N₂ with stirring at 25 °C.

Potassium superoxide reactivity

1–3 were allowed to react with one equivalent KO₂ in acetone/DMF (4:1 v/v). The stock solution of KO₂ (50 mM) was prepared in the same solvent system using equimolar amounts of 18-Crown-6 to solubilize KO₂. The reaction mixture was stirred at 25 °C.

Quantification of liberated hydrogen peroxide

Amount of H₂O₂ was determined by iodometry as follows. The nickel complexes **1–3** were reacted with two equivalents of HClO₄ followed by addition of one equivalent KO₂ in Acetone/DMF mixture (4:1 v/v) at room temperature (18-Crown-6 was used to solubilize KO₂). After completion of the reaction, another two equivalents of HClO₄ was added to stabilize the liberated H₂O₂. Then 1 mL of this final reaction mixture was diluted to 11 mL and was treated with excess NaI. The amount of I₃[−] liberated was quantified using UV-Vis spectroscopy (λ_{max} = 361 nm, ϵ = 2.5×10^4 M^{−1} cm^{−1})

RESULTS AND DISCUSSION

Synthesis

The synthesis of the pentadentate N5 ligands (Scheme 1) was accomplished following a route similar to published methods.^{46,47} The ligands TPMEN and PhTPEN were synthesized by mixing three equivalents of picolyl chloride hydrochloride with one equivalent of a diamine (N-methylethylenediamine and N-phenylethylenediamine respectively). The ligand TQMEN was obtained using N-methylethylenediamine and 2-(chloromethyl) quinoline hydrochloride in the place of picolyl chloride hydrochloride. Complex **1** was known in the literature and was synthesized by following the published procedure.⁴⁹ The nickel complexes **2–3** were prepared by adding one equivalent of ligand to a solution of [Ni(ClO₄)₂]•6H₂O in methanol. The complexes were characterized by various analytical and spectroscopic techniques.

Optical, electrochemical and magnetic properties of complexes

The nickel complexes **1, 2**, and **3** are soluble in polar solvents but sparingly soluble in non-polar solvents such as dichloromethane. Optical spectra for **1–3** in DMSO, acetonitrile and methanol are shown in Figure 3, with relevant electronic absorption parameters summarized in Table 1. Complexes **1** and **2** have similar spectra in MeCN, with a peak around 525 nm

and a broad peak in the near-IR spanning 800 nm to 1100 nm. However, substitution of the methyl group of the methylene bridge in **1** for a phenyl group in **2** results in a shift to lower energy ($\lambda = 545/904$ nm for **1** vs. $\lambda = 562/1041$ nm for **2**). This change in peak position is common in this type of ligand system and likely arises from the steric effect of the phenyl group.⁴⁹ Similar UV-visible absorption spectra have been reported for several five-coordinate Ni(II) complexes.^{37,50} Complex **3**, in contrast, showed very different optical spectra compared with **1** and **2**, exhibiting a broad peak at 676 nm in MeCN and 691 nm in MeOH. In addition, a distinct shoulder in the 400 – 500 nm region can be observed in both methanol and MeCN (Figure 3). The observed trend of red-shifting of wavelength when pyridine arms were substituted by quinoline is consistent with our previous observation in a different ligand system, and could be attributed to weakening of the ligand field upon introduction of the steric bulk.⁵¹ The optical parameters summarized in Table 1 are consistent with previously reported Ni complexes in octahedral geometry.^{49,52} Optical spectra of **1** in dichloromethane/acetonitrile (3:1 v/v) showed peaks at 525, 807, and 877 nm, and matched well with reported values.⁴⁹ The newly synthesized complexes **2** and **3** showed a reasonable trend in the optical properties. Based on the optical data, magnetic moment and X-ray absorption spectroscopy (*vide infra*) it can be concluded that all three complexes support Ni(II) in an octahedral geometry.

Solid state magnetic susceptibility measurements were performed on complexes **1**, **2** and **3** at room temperature; values for the effective magnetic moment and magnetic susceptibility are given in Table 1. For all three, the positive value of χ_A suggests that the nickel complexes are paramagnetic.⁴⁵ The μ_{eff} values of 2.82, 2.96, and 3.36 B.M. lie within the range expected for Ni(II) in an octahedral environment, and agree with results reported previously on similar Ni(II) complexes.^{53,54}

The reduction potential of complexes **1** – **3** were measured using cyclic voltammetry (Figures S1–S3). The complexes were scanned from –1.5 V to + 1.0 V in DMF with a scan rate varied from 100 mV/s to 500 mV/s. All three Ni complexes exhibited anodic waves corresponding to the Ni(II)/Ni(III) redox couple around –0.75 V versus Ag/AgCl (–0.75 V for **1**, –0.75 V for **2**, and –0.77 V for **3**). The Ni(II)/Ni(III) couples for all three complexes are almost identical despite the differences in the supporting ligand and well within the range of other known Ni complexes.⁵⁵ However, due to the non-reversible nature of the Ni(II)/Ni(III) couple in these Ni complexes, only reduction of superoxide without the subsequent oxidation of superoxide is expected. The pyridine complexes **1** and **2** exhibit reversible waves corresponding to Ni(I)/Ni(II) with an $E_{1/2} = -1.35$ V and –1.15, respectively. Complex **3** demonstrated a non-reversible anodic wave corresponding to Ni(I)/Ni(II) at –1.45 V.

Characterization of complex **1**, **2** and **3** using XAS

X-ray absorption spectroscopy (XAS) measurements were carried out to directly probe nickel oxidation state, site symmetry, and metrical coordination parameters. X-ray absorption near edge spectroscopy (XANES) data for **1** – **3**, shown in Figure 4 (also Figure S4), suggest very similar environments for the nickel centers in all three complexes, and are consistent with assignment to a nickel(II) state. For all three complexes, the pre-edge feature

at 8333.6 eV, associated with formally spin-forbidden $1s \rightarrow 3d$ transitions, is extremely weak, supporting a nickel(II) site having either O_h or D_{4h} symmetry.⁵⁶ The lack of any visible shoulder features along the rising edge associated with $1s \rightarrow 4p +$ shakedown transitions support assignment to six-coordinate octahedral nickel(II) centers in **1** – **3**, in accord with our electronic absorption and magnetic measurements.⁵⁶

Extended X-ray absorption fine structure (EXAFS) analyses, summarized in Figure 5 and Tables S1–S3, lend further credence to our XANES conclusions. All three complexes are best fit by a single shell of six ligating nitrogen atoms at 2.07–2.09 Å. The Ni-N bond distances obtained for complexes **1** – **3** match reasonably well with the Ni-N bond distances obtained from single crystal X-ray analysis of Ni(II) complexes in a similar ligand environment.^{49,52,57} It was not possible to resolve distinct sub-shells of nitrogen ligands due to the 0.13 Å resolution of the EXAFS data. We note that **2** exhibits a slightly shorter average bond length of 2.07 Å and a goodness-of-fit trend that more clearly favors a 5 to 6-coordinate solution compared to the 6 to 7-coordinate states and 2.09 Å bond lengths seen for **1** and **3**. This observation is in accord with the somewhat reduced intensity of the white line seen for **2** in the XANES spectra, relative to **1** and **3**. Additional contributions to the best fits include outer shell single and multiple-scattering pathways arising from the carbon atoms of the pyridine moieties and backbone carbons of the respective ligands.

Oxidation of complexes with Ferrocenium

The potential of these complexes toward stabilizing an oxidizing higher valent state was investigated by treating the complexes with ferrocenium hexafluorophosphate ($FcPF_6$) in DMSO. The reaction was monitored by optical spectroscopy while sub-stoichiometric amounts of $FcPF_6$ were added gradually to a solution of complex **1**. As the reaction progressed a peak developed at 368 nm, although it is masked under other contributions to the optical spectra. Optical spectra of **1** after treatment with 1 equivalent of $FcPF_6$ clearly showed a peak at 367 nm with a shoulder at 422 nm (Figure S5); in DMSO complex **1** has no peak in that region (Figure 3). Under the same conditions complex **2** and **3** developed peaks at 360 nm and 399 nm respectively (Figure S5). A recently reported Ni(II) complex with mixed N/O coordination was oxidized to Ni(III) via $FcPF_6$, with the Ni(III) species showing charge transfer bands at 380 and 450 nm, similar to our reported spectra.³⁷ The observation of a putative Ni(III) species through chemical oxidation (Figures S6 – S8) suggests that the reduction potentials of the complexes **1**, **2** and **3** are definitely lower than 0.505 V (referenced to Ag/AgCl) as observed for the Fc^+/Fc couple.⁵⁸

Reactivity with KO_2

The complexes **1** – **3** were also treated with potassium superoxide (KO_2) to test their reactivity toward superoxide. Addition of 10 equivalents of KO_2 in DMSO caused the nickel complexes to disintegrate. However, addition of a single equivalent of KO_2 in an acetone/DMF mixture (4:1 v/v) led to changes in optical spectra without disintegrating the complex. The spectral changes for the reaction of **1** with KO_2 are shown in Figure 6A. Upon addition of KO_2 , a new feature develops at 425 nm, while the peak associated with complex **1** at 541 nm also increases in intensity and the peak at 915 nm decreases slightly. After 45 minutes the feature at 425 nm diminished and the features at 541 nm and 915 nm showed

minor losses in intensity. The reaction of **2** and **3** with KO_2 followed a similar pattern (Figures 6B and 6C): a peak growth at 370–425 nm region, the peak at 550–670 nm increases its intensity, and the broadband peak in the 1000 nm range decreases its intensity. Moreover, these developing features rapidly disappeared at room temperature suggesting that these features are associated with a fleeting intermediate. These results are in accord with previously reported absorption spectra of a Ni(II)-superoxo intermediate, which showed an absorption feature at 425 nm along with a broad shoulder spanning 525–650 nm.³⁶ Based on the available literature precedent, our data also support presence of a fleeting intermediate reminiscent of a Ni(II)-superoxo intermediate, but further experimentation is needed to validate this claim.

The reaction of complexes **1** – **3** with FcPF_6 and KO_2 described previously (*vide supra*) was rather promising and led us to investigate the potential of these complexes toward superoxide reduction in a manner analogous to the first half reaction of native NiSOD (equation 1).

If complexes **1** – **3** are able to reduce superoxide, hydrogen peroxide (H_2O_2) production should be evident. Following a reported procedure, sodium iodide assays were employed to detect and quantify the amount of hydrogen peroxide produced by **1** – **3**.⁵⁹ The assays were done in the presence of perchloric acid (HClO_4) due to the stability it affords to any liberated H_2O_2 . All three complexes show evidence for H_2O_2 production by this assay (Figure S9), with comparable yields of H_2O_2 (~ 15 – 20%). However, the yield of H_2O_2 produced by complex **1** (18%) and **2** (19%) is slightly higher than that of **3** (15%). Control reactions in the absence of any nickel complexes showed no discernible H_2O_2 yield. Interestingly, when this reaction was monitored for complex **2** using optical spectroscopy (Figure S10), no peak growth at 424 nm region was observed. This indicate the probable nickel superoxo intermediate observed before with one equivalent KO_2 is either too short lived under these conditions to be detected optically or not forming in the presence of acid as the presence of proton source will facilitate superoxide reduction significantly.

Conclusion

In summary, this paper reports the synthesis and characterization of a family of Ni(II) complexes supported by polypyridyl ligands, and also demonstrated their potential to act as functional mimics of the oxidative phase of the reaction catalyzed by Ni-SOD. Complexes **1**, **2** and **3** were characterized by traditional synthetic and analytical techniques, as well as X-ray absorption spectroscopy. Optical spectroscopy suggested that all complexes showed reactivity toward superoxide suggesting formation of a fleeting intermediate, tentatively

described as a Ni(II)-superoxo adduct. This data was further substantiated by iodometric assays that demonstrated superoxide reduction to H₂O₂. To our knowledge this is the first set of Ni complexes supported by neutral polypyridyl ligands that showed superoxide reduction, thus mimicing the oxidative phase of the enzyme. Current work in the group is focused on low temperature kinetic studies to stabilize and trap the fleeting intermediate for spectroscopic characterization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Superoxide dismutase (SOD) enzymes catalyze the disproportionation of superoxide into hydrogen peroxide and molecular oxygen
- Three Nickel complexes were synthesized and characterized
- X-ray Absorption spectroscopy suggest Ni(II) in octahedral environment
- Complexes demonstrated superoxide reduction capabilities

Figure 1.
Active site of NiSOD.

Figure 2.

Nickel complexes used in this study: **1**, $[\text{Ni}(\text{TPMEN})(\text{ClO}_4)_2]$; **2**, $[\text{Ni}(\text{PhTPEN})(\text{ClO}_4)_2]$; and **3**, $[\text{Ni}(\text{TQMEN})(\text{ClO}_4)_2]$.

Figure 3.

UV-Visible spectra of complexes **1** (red), **2** (blue) and **3** (black) in polar solvents DMSO (Panel A), MeOH (Panel B) and MeCN (Panel C).

Figure 4. Normalized XANES spectra of complexes **1**, **2**, and **3**. The inset shows an expansion of the pre-edge region.

Figure 5.

Representative best fits (bolded entries in Tables S1–S3) to k^3 -weighted EXAFS data of **1**, **2**, and **3**. Experimental data is shown as a dotted line, while the best fit is shown as a solid line.

Figure 6.

Absorption spectra obtained upon reaction of complexes with 1 equivalent KO_2 in Acetone/DMF mixture (4:1 v/v) at RT; 1 cm pathlength. Starting material (black), spectrum immediately after addition of KO_2 (red), final spectrum after 45 minutes (blue) are shown in different panels **1** (A), **2** (B) and **3** (C).

Scheme 1.

Generic scheme for synthesis of the N5 ligands: A) TPMEN, B) PhTPEN, C) TQMEN

Table 1Optical and magnetic parameters of complexes **1** – **3**

Compound	Optical Parameters λ (nm), [ϵ ($M^{-1}cm^{-1}$)]			Magnetic Parameters	
	Methanol	Acetonitrile	Dimethyl Sulfoxide	μ_{eff} (B.M.)	χ_M ($emu\ mol^{-1}$)
1	545 [27], 795 [23], 904 [30]	522 [12], 862 [11]	537 [31], 808 [20], 927 [26]	2.82	3.34×10^{-3}
2	562 [19], 1041 [22]	532 [31], 822 [19], 953 [29]	555 [20], 1022 [21]	2.96	3.67×10^{-3}
3	409 [sh, 58], 471 [sh, 30], 693 [11]	417 [sh], 465 [sh], 676 [25]	443 [69], 470 [44], 712 [7]	3.36	4.75×10^{-3}