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Synthesis and Characterization of $Ni^{III}N3S2$ Complexes as Active Site Models for the Oxidized Form of Nickel Superoxide Dismutase

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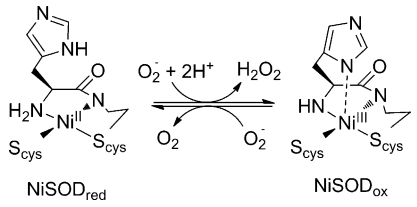
Abstract: Nickel complexes, $[Ni(H_2BA^RTPP)](ClO_4)_2$ (R = Ph for **1** or *i*Pr for **2**), supported by a pentadentate ligand H_2BA^RTPP were synthesized and oxidized to form Ni^{III} species having a N3S2 coordination environment to mimic the active site of the oxidized form of nickel superoxide dismutase (NiSODox). The Ni^{III} species $\mathbf{2}^+$ exhibited a rhombic signal with *g* values at 2.15, 2.12 and 2.02 similar to that of NiSODox. DFT calculations revealed that $\mathbf{2}^+$ has an unpaired electron primarily located in the d_{z^2} orbital of the Ni^{III} center, which strongly overlaps with the p_z orbital of the axial pyridine nitrogen of $H_2BA^{Pr}TPP$.

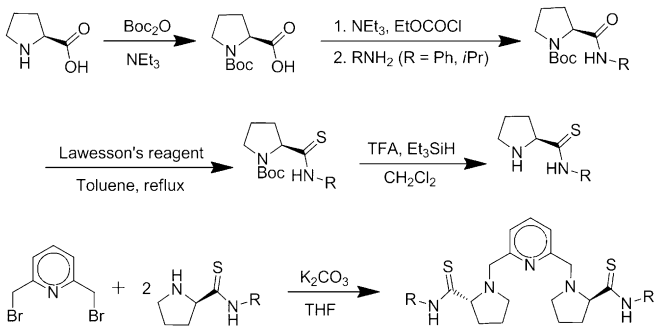
Nickel superoxide dismutase (NiSOD), a new and distinct class of SOD, was discovered from *Streptomyces*[1] and cyanobacteria.[2] The enzyme can catalyze the disproportionation of superoxide anion (O_2^-) to dioxygen (O_2) and hydrogen peroxide (H_2O_2) by cycling between Ni^{II} and Ni^{III} oxidation states. Numerous studies have shown that O_2^-, a cellular toxin produced by NADPH oxidase, can cause irreversible cellular damage[3] resulting in neurodegenerative diseases (e.g., Parkinson's[4] and Alzheimer's[5]), as well as cardiovascular diseases[6] and some cancers. The protein structure of the reduced form of NiSOD ($NiSOD_{red}$), which has been recently determined, reveals the nickel active site containing one bound nitrogen of the N-terminal amine of His1, the other bound nitrogen from the backbone amide of Cys2, two bound cysteinate (S^-) residues from Cys2 and Cys6 on the equatorial plane, and the imidazole residue of His1 at the position proximal to the nickel center (Scheme 1).[7] The oxidized form of NiSOD ($NiSOD_{ox}$) possesses an axial nitrogen from His1 that is coordinated to the Ni^{III} center to form a distorted square pyramidal active site ($\tau = 0.20$). The $NiSOD_{ox}$ has a rhombic electron spin resonance (EPR) spectrum with a low-spin ($S = 1/2$) Ni^{III} signal ($g_x = 2.30$, $g_y = 2.24$, and $g_z = 2.01$ with $A_{zz} = 24.9$ G).



Scheme 1. The disproportionation of O_2^- into O_2 and H_2O_2 through a cycle of Ni^{II} and Ni^{III} oxidation states.

Recently, several model compounds with N2S2 square planar geometry for the active site of $NiSOD_{red}$ have been synthesized.[8] Maroney et al. have demonstrated that the axial imidazole plays an essential role in optimizing the rate of O_2^- disproportionation.[9] Therefore, mimics with an N3S2 coordination environment of the active site of NiSOD will be critical for the study of the role of axial bound histidine in $NiSOD_{ox}$. Herein, we present two novel Ni^{III} complexes, supported by an N3S2 ligand, which closely mimic the active site of $NiSOD_{ox}$. The structures and electronic properties of these two model compounds provide insight into the active site of $NiSOD_{ox}$.

To construct nickel complexes with an N3S2 coordination environment that mimic the active site of $NiSOD_{ox}$, two ligands, 2,6-bis((2-(*N*-anilinylthiocarbonyl)pyrrolidin-1-yl)methyl)pyridine ($H_2BA^{Ph}TPP$) and 2,6-bis((2-(*N*-isopropylaminothiocarbonyl)pyrrolidin-1-yl)methyl)pyridine ($H_2BA^{Pr}TPP$) were designed and synthesized partially using a method found in the literature (Scheme 2).[10] The reaction of $H_2BA^{Ph}TPP$ with $[Ni(CH_3CN)_6](ClO_4)_2$ in CH_3CN gave a five-coordinate Ni^{II} complex,



Scheme 2. Synthetic procedure of H_2BA^RTPP (R = Ph or *i*Pr).

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$[Ni(H_2BA^{Ph}TPP)](ClO_4)_2$ (**1**). An analogue, $[Ni(H_2BA^{Pr}TPP)](ClO_4)_2$ (**2**), was also synthesized using $H_2BA^{Pr}TPP$ as the supporting ligand. Crystals of **1** and **2** were obtained by slow diffusion over two days at ambient temperature. The molecular structures of **1** and **2** determined by X-ray crystallography reveal that the Ni^{II} center of **1** and **2** is coordinated by the pentadentate $H_2BA^{R}TPP$ (R = Ph or *i*Pr) forming a NiN3S2 arrangement in a C_2 symmetric fashion (Figure 1). The two bound thioamide

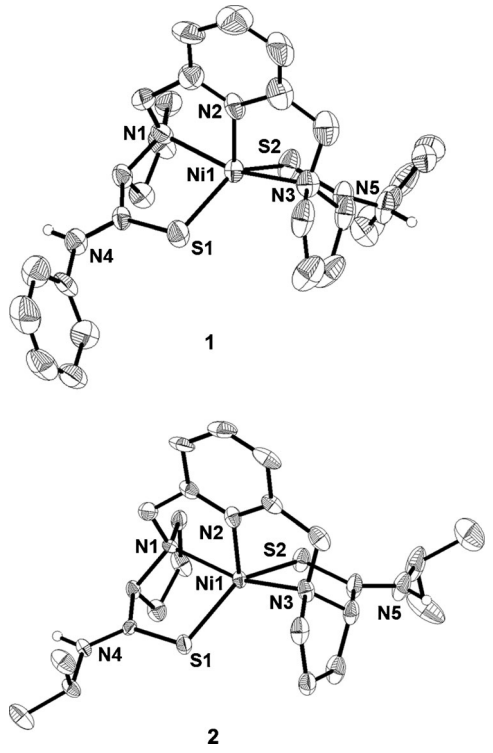


Figure 1. Thermal ellipsoid representation of **1** and **2** at 50% probability level. Hydrogen atoms excepting thioamide protons and counter ions of **1** and **2** are omitted for clarity.

S atoms of $H_2BA^{R}TPP$ on complexes **1** and **2** are *trans* to each other with a S-Ni-S angle of 136.3° and 143.4°, respectively. The τ values (0.41 and 0.29) of five-coordinate complexes **1** and **2** suggest that the geometry of **1** and **2** is distorted square pyramidal.[11] The metric parameters of **1** and **2** are presented in Table 1.

Table 1. Selected bond lengths [Å] and angles [°] for **1** and **2**.

	1	2
Ni(1)-S(1)	2.3215(11)	2.333(3)
Ni(1)-S(2)	2.3152(12)	2.325(3)
Ni(1)-N(1)	2.171(4)	2.155(8)
Ni(1)-N(2)	1.949(4)	1.953(8)
Ni(1)-N(3)	2.150(4)	2.146(8)
N(2)-Ni(1)-N(1)	80.80(16)	80.2(3)
N(2)-Ni(1)-N(3)	80.16(15)	81.1(4)
N(2)-Ni(1)-S(1)	113.04(12)	110.4(2)
N(2)-Ni(1)-S(2)	110.62(12)	106.2(3)
S(1)-Ni(1)-S(2)	136.34(5)	143.46(13)
N(1)-Ni(1)-N(3)	160.94(14)	161.4(3)

The UV/Vis spectrum of **1** (Figure S1 in the Supporting Information) has two characteristic absorption bands at 310 and 355 nm (15800 and 9220 $M^{-1}cm^{-1}$) and two d–d transition bands at 470 (shoulder, 520 $M^{-1}cm^{-1}$) and 645 nm (200 $M^{-1}cm^{-1}$). The UV/Vis spectrum of **2** (Figure S2 in the Supporting Information), which is similar to the spectrum of our previously reported Ni^{II}(BDPP) complex,[12] exhibits a characteristic absorption band at 360 nm (3600 $M^{-1}cm^{-1}$) and two d–d transition bands at 460 and 655 nm (90 and 120 $M^{-1}cm^{-1}$). Cyclic voltammograms of **1** and **2** exhibit quasireversible and reversible curves with $E_{1/2}$ values of 0.837 and 0.836 V versus Fc/Fc^+ in CH_2Cl_2, respectively (Figure 2, inset). The quasireversible behavior of **1** can be attributed to the partial dissociation of thioamide protons of $H_2BA^{Ph}TPP$, due to the increase in acidity upon the oxidation of **1**. The electrochemical study suggests that complexes **1** and **2** can be oxidized to Ni^{III} species mimicking the oxidized form of NiSOD.

Notably, treatment of **2** with the oxidant $(NH_4)_2Ce(NO_3)_6$ in THF immediately changed its color from green to brown, indicating the formation of $\mathbf{2}^+$. The UV/Vis spectrum of $\mathbf{2}^+$, obtained from the reaction solution (Figure 2), displayed two CT bands at 310 and 360 nm (7000 and 3200 $M^{-1}cm^{-1}$) and a d–d transition band at 480 nm (700 $M^{-1}cm^{-1}$). The UV/Vis absorption pattern of the oxidized product $\mathbf{2}^+$ is comparable to those of as-isolated $NiSOD_{ox}$ (372 and 507 nm)[13] and reported $[Ni^{III}(BDPP)](PF_6)$ (300, 380, and 450 nm)[12] However, treatment of **1** with $(NH_4)_2Ce(NO_3)_6$ in THF resulted in a decomposition reaction observed by UV/Vis spectroscopy.

The EPR spectrum of $\mathbf{2}^+$, generated in situ in THF at 77 K, exhibited a rhombic signal that confirmed the formation of a square pyramidal Ni^{III} species with *g* values of 2.15, 2.12 and 2.02 (Figure 3). The observed EPR signal was similar to that of $NiSOD_{ox}$ ($g_x = 2.30$, $g_y = 2.24$, and $g_z = 2.01$). Notably, only few Ni^{III} species in a distorted square pyramidal ligand environment have been reported in the literature.[14] The EPR spectrum of the reaction mixture of **1** and $(NH_4)_2Ce(NO_3)_6$ was also obtained, but only a very weak Ni^{III} signal was observed (Figure S5 in the Supporting Information). Recently, Harrop et al. reported a $NiSOD_{ox}$ active site mimic that contained an N_3S_2 ligand. The EPR spectrum of their oxidized products, generated in situ, revealed a thiyl radical signal with a *g* value of 2.00 and an anisotropic signal ($g = 2.26$, 2.17, ~2.00) for Ni^{III} species.[15]

Complexes **1** and **2** were individually added to CH_3CN suspensions of KO_2 to evaluate the probability of the oxidation of **1** and **2** by O_2^- anion. Unfortunately, neither Ni^{III} species nor disproportionation products of O_2^- were formed. Instead, deprotonated products $Ni(BA^{Ph}TPP)$ (**3**) and $Ni(BA^{Pr}TPP)$ (**4**) were obtained. Complexes **3** and **4** could also be obtained by adding NEt_3 to **1** and NaH to **2**, respectively, to remove the thioamide protons of the corresponding ligand to form imidothiolate groups of $BA^{R}TPP^{2-}$ (R = Ph or *i*Pr) in **3** and **4** (Scheme 3). Recrystallization of **3** and **4** by slow diffusion gave green needle-like crystals. X-ray analysis revealed that complexes **3** and **4** are five-coordinate Ni^{II} species (Figure S6 in the Supporting Information) with τ values (0.27 for **3** and 0.32 for **4**) comparable to that of $NiSOD_{ox}$ ($\tau = 0.20$). With a dianionic supporting ligand, $BA^{R}TPP^{2-}$ (R = Ph or *i*Pr), complexes **3** and **4**

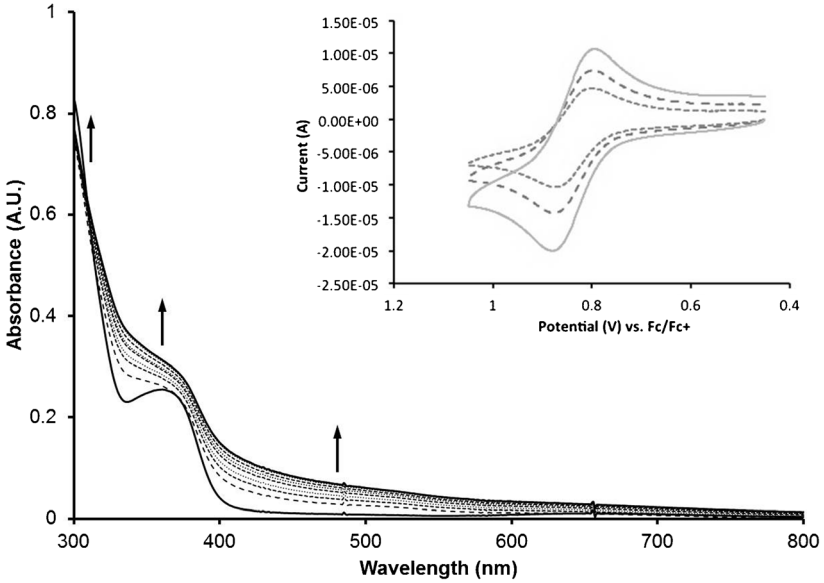


Figure 2. Oxidation experiment of **2** in THF using $(NH_4)_2Ce(NO_3)_6$ monitored by UV/Vis spectroscopy. Inset: cyclic voltammograms of **2** in CH_2Cl_2 with different scan rates.

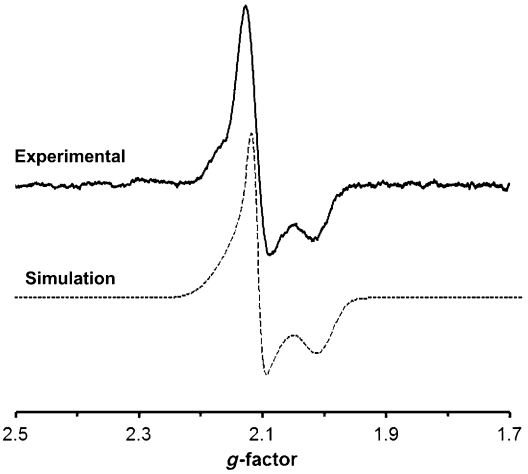
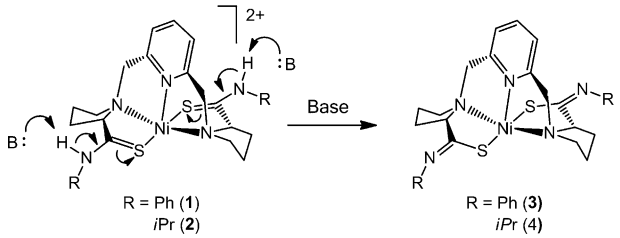


Figure 3. X-band EPR spectra of $\mathbf{2}^+$ recorded in frozen THF at 77 K.



Scheme 3. Deprotonation of **1** and **2** by NEt_3 and NaH, respectively, to form **3** and **4**.

are suspected to be readily oxidized to Ni^{III} species. However, the cyclic voltammogram of **3** revealed two irreversible oxidation events at 352 and 630 mV versus Fc/Fc^+ in CH_2Cl_2, and that of **4** revealed such events at 334 and 637 mV (Figure S7 in the Supporting Information). The irreversible behavior of **3** and **4** observed in cyclic voltammetry is similar to that of most model compounds for $NiSOD_{red}$.[8f,16] Consequently, no Ni^{III} species was observed from the reaction of **3** or **4** with $(NH_4)_2Ce(NO_3)_6$.

A DFT calculation for $\mathbf{2}^+$ was performed to gain insight into the geometric and electronic structure of the Ni^{III} center. The geometry of the Ni^{III} center in the optimized structure of $\mathbf{2}^+$ in the gas phase reveals a distorted square pyramidal ligand environment with S-Ni-S and N_{eq}-Ni-N_{eq} angles of 137.73° and 165.71°, respectively, and a τ value of 0.47. The average calculated lengths of Ni–N_{eq} and Ni–S are 2.053 and 2.359 Å, respectively, and that of Ni–N_{ax} is 1.929 Å. Owing to the rigidity of $H_2BA^{Pr}TPP$ in comparison with the coordinated residues in the active site of $NiSOD_{ox}$, the Ni–N_{ax} length of $\mathbf{2}^+$ is shorter than the Ni–N_{His} length of $NiSOD_{ox}$ (2.35 Å), as a result, the Ni–N_{eq} and Ni–S lengths of $\mathbf{2}^+$ slightly exceed those of $NiSOD_{ox}$ (1.965 Å for Ni–N_{eq} and 2.175 Å for Ni–S_{Cys}). The spin density plot of $\mathbf{2}^+$ shows that the Ni^{III} center has an unpaired electron primarily located in the d_{z^2} orbital of the Ni^{III} center, which strongly overlaps the p_z orbital of the axial pyridine nitrogen of $H_2BA^{Pr}TPP$ (Figure 4b). The DFT calculation also reveals that complex **2** is readily oxidized to Ni^{III} species due to the strongly coordinated pyridyl group in the axial position and the electron-rich isopropyl thioamide groups on the equatorial plane. In contrast, the spin density plot of $\mathbf{1}^+$ (Figure 4a) shows that an unpaired electron can delocalize to the phenyl ring of $H_2BA^{Ph}TPP$. The delocalization of the unpaired electron could cause side reactions to occur on the

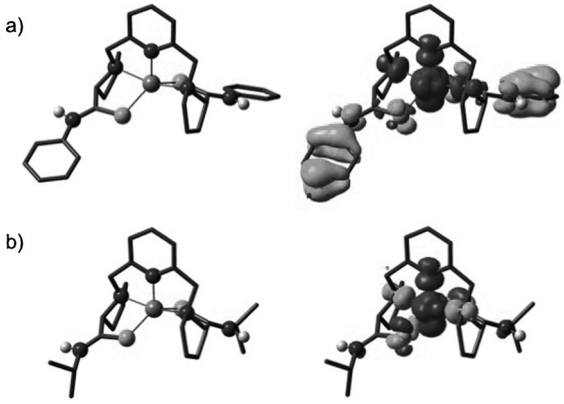


Figure 4. Optimized gas-phase structure (left) and spin density (right) for: a) $\mathbf{1}^+$, and b) $\mathbf{2}^+$, derived from DFT calculations.

phenyl ring or the thioamide nitrogen of $H_2BA^{Ph}TPP$ leading to the decomposition of $\mathbf{1}^+$. Such decomposition was observed upon the oxidation of **1** by $(NH_4)_2Ce(NO_3)_6$ by UV/Vis and EPR spectroscopy. In addition, the cyclic voltammograms of **1** in different scan rates exhibited that more oxidized $\mathbf{1}^+$ would decompose when the scan rate was decreased (Figure S9 in the Supporting Information).

In conclusion, two Ni^{II} complexes **1** and **2**, which can be oxidized to Ni^{III} species to mimic the active site of $NiSOD_{ox}$, were synthesized. With the axial pyridyl group and electron donating isopropyl thioamide groups, complex **2** was oxidized by $(NH_4)_2Ce(NO_3)_6$ to form a metastable Ni^{III} species, which exhibited a rhombic signal in its EPR spectrum. Also, DFT calculations supported the formation of $\mathbf{2}^+$. The Ni^{III} complex $\mathbf{2}^+$ has a N3S2 ligand environment closely mimicking the first coordination sphere of the active site of $NiSOD_{ox}$. Furthermore, our results suggest that the axial His1 of NiSOD plays an important role in stabilizing the Ni^{III} center of the oxidized form of NiSOD. However, the reduction potential of **2** (0.836 V vs. Fc/Fc^+ in CH_2Cl_2) is much higher than that of wild-type NiSOD at −0.365 V versus Fc/Fc^+ in aqueous solution[17] lying between the reduction of dioxygen (−0.560 V vs. Fc/Fc^+) and superoxide anion (0.470 V vs. Fc/Fc^+).[18] Therefore, no SOD activity was observed for complex **2** even it can be oxidized to $\mathbf{2}^+$, a Ni^{III} complex. Efforts are underway in our laboratory to prepare appropriate pentadentate ligands in order to construct stable NiSOD mimics, which have the reduction potentials between the reduction of dioxygen and superoxide anion, possessing the ability of catalyzing the dismutation of O_2^-.

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Keywords: biomimetics · catalysis · density functional calculations · nickel(III) complexes · superoxide

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