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Versatile Methodology Toward NiN_2S_2 Complexes as Nickel Superoxide Dismutase Models: Structure and Proton Affinity

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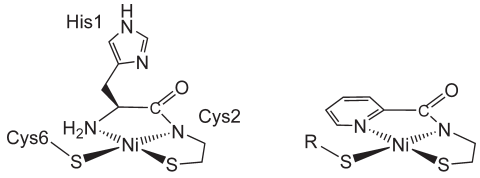
Structural features of the reduced form of the nickel superoxide dismutase (Ni-SOD) active site have been modeled with asymmetric NiN_2S_2 complexes (Et_4N)[Ni(nmp)(SR)] (R = C_6H_4-*p*-Cl (**2**) and (S^tBu) (**3**)) obtained via S,S-bridge splitting of the dimeric metallosynthon, $[Ni_2(nmp)_2]$ (**1**). Complexes **2** and **3** are irreversibly oxidized at potentials within the window needed for SOD activity, 236 and 75 mV versus Ag/AgCl, respectively. The exogenous thiolato-S in **2** and **3** serves as a proton acceptor, suggesting potential involvement of Cys6 in Ni-SOD for H^+ storage between SOD half reactions.

The reactive oxygen species (ROS) superoxide ($O_2^{\bullet-}$) is an inevitable cytotoxic byproduct of aerobic metabolism, which if not eliminated can lead to a variety of health disorders.[1] To combat this ROS, all aerobic organisms possess metalloenzymes known as superoxide dismutases (SODs) that catalyze the disproportionation of $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2) and O_2 through alternate oxidation and reduction of their catalytic metal centers.[2] These SODs have been extensively characterized and employ metal cofactors such as Fe,[3,4] Mn,[5] or Cu/Zn[6] to catalyze the disproportionation reaction. Recently, a new class of SODs containing Ni (Ni-SOD) has been characterized; however, less is known regarding the mechanism of this SOD.[7,8] The overall protein fold, spectroscopy, ligands, and coordination geometry in Ni-SOD are quite distinct from those of other SODs. For example, Ni-SOD in its reduced Ni^{II} form ($Ni\text{-}SOD_{red}$) is ligated in an N_2S_2 square-planar geometry arising from the primary amine-N of His1, carboxamido-N from Cys2, and two thiolato-S donors from Cys2 and Cys6 (Chart 1). The presence of carboxamido-N and primary amine-N provides an unusual set of donors and raises questions with regard to the properties they impart on the Ni center, with few examples known in biology.[9,10] Another striking feature of Ni-SOD is the presence of two coordinated cysteine thiolates, which are themselves subject to oxidation in the presence of ROS.[11] Although the highly covalent nature of thiolate ligation is believed to aid in modulating the Ni^{II}/Ni^{III} redox couple to a value suitable for physiological function,[12] the interactions directing metal-based redox are not yet fully understood.[13,14] Additional questions arise as to how superoxide coordinates, the source of protons in the oxidative half-reaction, and how these tie into the overall Ni-SOD catalytic mechanism.

The report of Ni-SOD has inspired us and others[15] to answer these questions through small molecule analogues in

Chart 1. Active Site of $Ni\text{-}SOD_{red}$ and the Model Complexes, [Ni(nmp)(SR)]$^-$, Used in This Study (R = C_6H_4-*p*-Cl (**2**) or ^{t}Bu (**3**))



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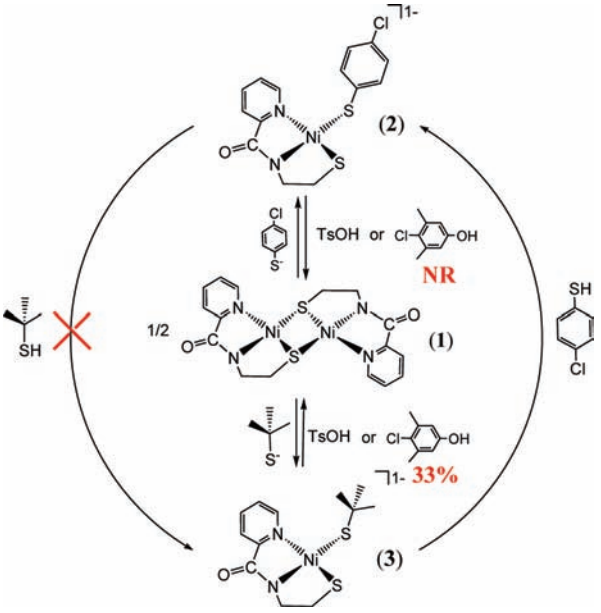
Scheme 1



order to investigate the physical properties of the models and shed light into the intrinsic properties of the Ni active site. Models employing both electronically accurate and asymmetric N_2S_2 spatial disposition are also quite scarce.[15] Herein, we report our initial work in this area, namely, the synthesis, structural and spectroscopic/electrochemical characterization, and proton binding studies of an unprecedented set of four-coordinate square-planar $Ni^{II}N_2S_2$ complexes as biomimetic structural analogues of Ni-SOD employing the tridentate ligand *N*-(2-mercaptoethyl)picolinamide ($nmpH_2$, where H represents dissociable protons; Chart 1).

When a DMF solution of nmp^{2-} is mixed with 1 mol equiv of $[Ni(H_2O)_6](ClO_4)_2$ under anaerobic conditions, a red-orange precipitate forms at room-temp (RT) consistent with the dinuclear S,S-bridged species, $[Ni_2(nmp)_2]$ (**1**) (Scheme 1). Since similar S,S-bridged species have been shown to break in the presence of strong ligands like CN^-,[16] we attempted bridge-splitting reactions with exogenous thiolate ligands to generate monomeric NiN_2S_2 complexes with an asymmetric coordination sphere, as observed in Ni-SOD. The addition of 2 mol equiv of RS^- (where $R = C_6H_4$-*p*-Cl or ^{t}Bu) to **1** resulted in formation of the corresponding mononuclear Ni^{II} complexes $(Et_4N)[Ni(nmp)(SC_6H_4$-*p*-$Cl)]$ (**2**) and $(Et_4N)[Ni(nmp)(S^tBu)]$ (**3**) in 70% yield for both (Scheme 1, Chart 1). The nmp^{2-} ligand replicates the two five-member chelate rings formed by His1 and Cys2 in Ni-SOD, while the exogenous thiolate allows for unconstrained modeling of Cys6.[8] Collectively, the bridge-splitting reaction of metallosynthon **1** with electronically different thiolate ligands provides a new, versatile, and general synthetic methodology for the preparation of monomeric and asymmetric NiN_2S_2 complexes that can be controlled and varied at the fourth coordination position.

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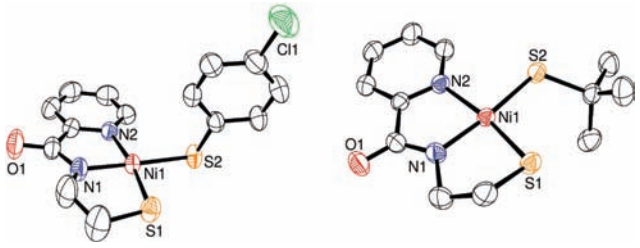

Figure 1. ORTEP diagrams of the anions of $(Et_4N)[Ni(nmp)(SC_6H_4$-*p*-$Cl)]$ (**2**) (left) and $(Et_4N)[Ni(nmp)(S^tBu)]$ (**3**) (right) showing 50% thermal ellipsoids for all non-hydrogen atoms. Selected bond distances (Å) and angles (deg) for **2** and **3**, values for **3** are shown in brackets: Ni1−N1, 1.8638(14) [1.882(2)]; Ni1−N2, 1.9470(14) [1.9635(19)]; Ni1−S1, 2.1492 (5) [2.1629(7)]; Ni1−S2, 2.2139(4) [2.1938(7)]; N1−Ni1−N2, 83.25(6) [82.30(8)]; N1−Ni1−S2, 176.73(5) [174.21(6)]; S1−Ni1−S2, 90.45(2) [98.14(3)].

Dark red X-ray quality crystals of **2** and **3** were obtained under anaerobic conditions by slow diffusion of Et_2O into THF solutions of the complexes at RT. The structures revealed four-coordinate square-planar Ni^{II} centers arising from the tridentate nmp^{2-} ligand and monodentate exogenous thiolate completing the N_2S_2 coordination sphere (Figure 1). The Ni−N/S distances are somewhat variable and in line with the electronic nature of the donor atom, as the $Ni-N_{amide}$ bond is shorter (1.8638(14) Å for **2** and 1.882(2) Å for **3**) than the $Ni-N_{py}$ bond in both **2** (1.9470(14) Å) and **3** (1.9635(19) Å), which results in a relative lengthening of the Ni−S bond that is trans to the carboxamido-N (2.2139(4) Å for **2** and 2.1938(7) Å for **3**). Slight distortions from a perfect square plane are also evident from the bond angles about Ni, with the most acute arising from the tight bite of the pyridine-2-carboxamide unit (Supporting Information for further structural information). The observed metric parameters for **2** and **3** are similar to those of other reported Ni^{II} complexes in similar coordination spheres[9,11,15] and are also strikingly similar to those determined for $Ni\text{-}SOD_{red}$,[8] presumably arising from the close match between donor type and spatial disposition provided by our model system (see Table S3, Supporting Information).

As described above, both **2** and **3** exhibit square-planar geometries in the solid state that also appear to be the same in solution. This property is clearly demonstrated in their ^{1}H NMR spectra, which display no paramagnetically shifted resonances expected for tetrahedral or octahedral geometry. This diamagnetism is not solvent dependent, as the square-planar ($S = 0$) state persists in both donor ($(CD_3)_2CO$) and non-donor ($CDCl_3$, THF-d_8) solvents (Supporting Information). The red-orange color of solutions of **2** and **3** result from presumed S-to-Ni charge transfer bands in their UV−vis spectra and are similar to other $Ni^{II}N_2S_2$ complexes[9,15,17] with bands at 450 nm (ε = 5450 M^{-1} cm^{-1}) and 464 nm (ε = 4540 M^{-1} cm^{-1}) for **2** and **3**, respectively (Figure 2). These λ_{max} values are similar to the visible band of $Ni\text{-}SOD_{red}$, which is also centered near 450 nm.[12] Complexes **2** and **3** are stable in the solid state upon exposure to air while solutions appear moderately stable over the course of hours as monitored by UV−vis spectroscopy.

Cyclic voltammetric (CV) measurements of **2** and **3** in MeCN display irreversible oxidation events at 236 and 75 mV versus Ag/AgCl, respectively. Their differences are largely

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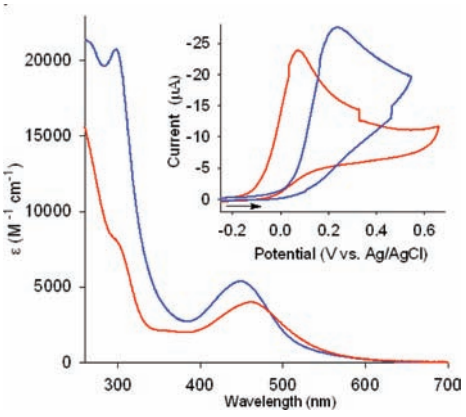

Figure 2. UV−vis spectra of **2** (blue) and **3** (red) in MeCN at 298 K. Inset: CV of 5 mM solutions of **2** (blue) and **3** (red) (vs Ag/AgCl in MeCN, 0.1 M $^{n}Bu_4NPF_6$ supporting electrolyte, glassy carbon working electrode, scan rate: 100 mV/s, RT).

attributed to the coordination of the more basic thiolate in **3** versus **2**. As expected, these values fall in between analogous $Ni^{II}N_2S_2$ complexes containing diamine-dithiolate[18] and dicarboxamide-dithiolate ligands.[17] While the oxidation potentials observed for **2** and **3** are intermediate between the aforementioned congeners and within the window acceptable for SOD chemistry,[19] the irreversible behavior is more similar to that of the bis-amine complexes. Bulk oxidation of MeCN solutions of **2** and **3** with a chemical oxidant like ferrocenium resulted in the partial formation of **1** and the corresponding disulfide, suggesting that the observed redox chemistry is in part ligand-based. It has been proposed that the N_2S_2 ligand environment alone in Ni-SOD is insufficient for attaining Ni^{III}, and stabilization is imparted from other interactions like coordination of the axial imidazole-N of His1 as observed in the Ni-SOD_{ox} structure.[8,12,15b] The addition of excess amounts (10 mol equiv) of potential N-donors such as N-methylimidazole or pyridine showed no observable change in the CV and UV−vis spectra of MeCN solutions of **2** and **3**, suggesting no affinity for these N-donors. The immediate proximity of His1 to the Ni center in Ni-SOD, however, may lead to immediate imidazole binding during turnover, whereas our systems do not benefit from any enhanced proximity via a built-in N-donor.

In addition to the binding of His1, another element in regard to the mechanism of Ni-SOD is the source of protons during the oxidative half reaction. One potential source may arise from the thiolate-S of Cys2 or Cys6. Indeed, protonation of Cys-S in Ni-SOD has been detected by S K-edge X-ray absorption spectroscopy and also supported by DFT calculations.[20] Thiolate protonation has also been suggested to prevent sulfur oxidation via removal of S(π) interactions from the HOMO.[13] In this regard, the capabilities of **2** and **3** as proton acceptors were tested. The addition of 1 mol equiv of tosic acid to **2** and **3** leads to near quantitative formation of **1** via protonation of the exogenous thiolate (Scheme 1). No decomposition of the Ni(nmp) unit resulted from this strong acid, further underscoring the stability of the coordinated tridentate ligand and suggesting that Cys6 may be the most likely candidate for protonation in Ni-SOD.[20c] The exogenous thiolate in these complexes can also be exchanged by the addition of a more acidic thiol. Treatment of **3** with 1 mol equiv of HSC_6H_4-*p*-Cl ($pK_a = 5.97$) afforded **2** in quantitative yield (Scheme 1). In contrast, the addition of 1 mol equiv of ^{t}BuSH ($pK_a = 17.9$) to **2** displayed no reaction (Scheme 1). Another proposed source of protons for the oxidative half reaction is Tyr9,[21] and thus we tested the proton-accepting ability of **2** and **3** with 4-chloro-3,5-dimethylphenol ($pK_a \sim 18$). No proton exchange was observed when 1 mol equiv of this phenol was added to **2**, consistent with our observation that proton exchange is largely governed by the pK_a. When 1 mol equiv of phenol was added to **3**, however, 33% of dimer **1** was generated. Although it may be expected that an equal distribution of **1** and phenol/thiol would be present on the basis of similar pK_a values, one must also consider the thiophilicity of Ni^{II} (borderline hard−soft acid), which supports the observed reaction path and lack of $[Ni(nmp)(OPh)]^-$ complex formation.

In summary, we have prepared two new complexes which successfully mimic electronically and spatially the primary coordination sphere of Ni-SOD_{red}. In doing so, we introduce a novel approach toward the preparation of NiN_2S_2 complexes with variable exogenous thiolate ligands that opens the door for the potential preparation of a large library of Ni-SOD synthetic analogues. Complexes **2** and **3** show irreversible oxidation events within the range necessary for SOD chemistry. However, the irreversibility suggests that the square-planar N_2S_2 donor set alone is insufficient to support Ni^{II}/Ni^{III} redox cycling, and stabilization is aided via another interaction like coordination of an axial N ligand. The reported complexes are the first to demonstrate a propensity to accept protons at the exogenous thiolato-S site in the presence of an appropriate proton donor, supporting a role for the cysteine thiolate(s) in proton storage and transport during SOD catalysis. The protonation of **3**, featuring a more "cysteine-like" alkyl thiolate, supports the likelihood of Tyr9 as a possible proton source in Ni-SOD. Recent studies on Tyr9 mutants by Maroney and co-workers demonstrate a decrease in activity and H_2O_2 saturation kinetics not observed for wild-type enzyme, highlighting its importance in catalysis and proposed role to aid in the release of H_2O_2.[21]

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Supporting Information Available: Experimental details and characterization of the ligand and complexes including tables and CIF files. This material is available free of charge via the Internet at http://pubs.acs.org.

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