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Synthesis and Sulfur Oxygenation of a (N_3S)Ni Complex Related to Nickel-Containing Superoxide Dismutase

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A nickel(II) thiolate complex incorporating three N-donor types (amino, amido, and imidazole) has been synthesized and characterized. The (N_3S)Ni complex, [*N*-{2-[(2-mercapto-2-methylpropyl)amino]ethyl}-1-methylimidazole-2-carboxamido]nickel(II) (**1**), is stable in the presence of O_2 but readily forms the sulfinato (RSO_2^-) derivative **2** upon the addition of H_2O_2. Electrochemical investigations of **1** reveal an irreversible sulfur-based oxidation at +0.17 V vs Fc^+/Fc (200 mV/s) that shifts to +0.81 V upon oxidation to **2**. Density functional theory investigations of **1** reveal a highest occupied molecular orbital that is predominantly sulfur-based, consistent with the observed sulfur-based oxidation and O_2 stability.

Catalytic disproportionation of superoxide to O_2 and H_2O_2 is a critical process catalyzed by one of several superoxide dismutase (SOD) metalloenzymes.[1] While most SODs employ N/O donor sets, an N/S core is found in NiSOD.[2−6] Reduced NiSOD ($NiSOD_{red}$) contains a square-planar nickel(II) coordinated by two thiolates (Cys-6/Cys-2), a carboxamido nitrogen (Cys-2), and the terminal amine of His-1 (Figure 1).[4] Oxidized NiSOD ($NiSOD_{ox}$) additionally binds the imidazole of His-1,[5,6] which may remain bound during catalytic turnover.[7,8] Although nickel thiolates are well-known to react with O_2 and H_2O_2, yielding sulfur-oxygenated derivatives,[9−13] no evidence of sulfur oxygenation has been observed in NiSOD.[9−13] It has been suggested by us and others that protonation and/or N−H···S hydrogen bonding may suppress sulfur oxygenation.[4,14−16] Herein we report the synthesis and characterization of a (N_3S)Ni complex (**1**) and its sulfinato derivative (**2**). Complex **1** is similar to a series of diamino (N_3S)Ni complexes recently reported by Darensbourg and co-workers with two notable distinctions.[17] First, **1** contains an amido donor in place of the second amine. Second, the chelate structure of **1** forces the in-plane imidazole parallel with the N_3S-donor plane, in contrast to a perpendicular or fluxional orientation in the complexes reported by Darensbourg et al. Although these complexes do not reproduce the axial histidine coordination of $NiSOD_{ox}$, they, nonetheless, offer insight into the reactivity of nickel-containing biomolecules.

The N_3S ligand, *N*-{2-[(2-mercapto-2-methylpropyl)amino]ethyl}-1-methylimidazole-2-carboxamide is readily prepared from isobutylene sulfide and *N*-(2-aminoethyl)-1-methylimidazole-2-carboxamide by the modification of known methods.[18−21] The addition of $Ni(acac)_2$ yields **1** as a red solid. The electronic spectrum of **1** (Figure S1 in the Supporting Information) displays bands at 356, 425, and 565 nm in acetonitrile and 367, 445(sh), and 573 nm in an aqueous

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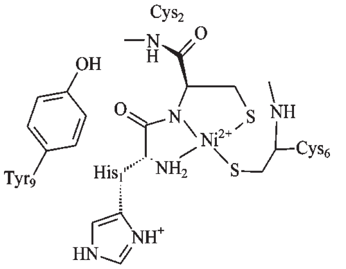
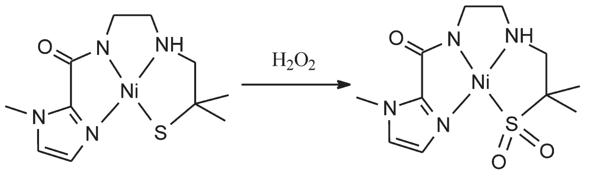


Figure 1. Representation of the active site of $\mathrm{NiSOD_{red}}$.

Scheme 1. Sulfur Oxygenation of **1** to **2**



1 **2**

solution. These features reproduce those observed in $\mathrm{NiSOD_{red}}$ (342, 361, 450, 488, 542, and 584 nm) and the maquette $\mathrm{NiSOD^{M1}}$ (337, 458, and 552 nm).[7,22]

Solutions of **1** are stable to O_2 (1 atm) in water, methanol, and acetonitrile with no detectable changes in the UV−visible spectrum or voltammogram after 2 weeks. This is consistent with the reported stability of $\mathrm{NiSOD_{red}}$ and in stark contrast to that of $\mathrm{NiSOD^{M1}}$, which decomposes within hours upon air exposure.[7] While **1** is O_2-tolerant, it reacts with H_2O_2 to yield the sulfinato derivative **2** (Scheme 1). The absorption bands of **2** (acetonitrile) are blue-shifted relative to **1** with a maximum at 315 nm and a shoulder near 400 nm (Figure S2 in the Supporting Information). The lower-energy band characteristic of **1** is notably absent. The IR spectrum of **2** displays intense bands at 1171 and 1042 $\mathrm{cm^{-1}}$ assigned as the symmetric and asymmetric S=O stretching frequencies of the sulfinato moiety (Figure S3 in the Supporting Information).[9−12]

Functional SOD mimics require a reversible redox couple between −0.3 and +0.9 V vs the normal hydrogen electrode (NHE).[23,24] In acetonitrile, **1** is oxidized at +0.17 V vs $\mathrm{Fc^+/Fc}$ (+0.86 V vs NHE).[25] The oxidation lies near the anodic limit for SOD mimics and is irreversible. Because protonation and/or N−H···S hydrogen bonding may be important at the NiSOD active site, voltammograms of **1** were also recorded in buffered aqueous solutions. The oxidation potential remains near the functional limit with values of +0.76, +0.80, and +0.85 V (vs NHE) at pHs of 9.1, 7.0, and 5.0, respectively, but with no change in reversibility. Previously, Darensbourg et al. reported that sulfur modification, including oxygenation and alkylation of nickel thiolates, can impart redox reversibility, albeit with a large anodic potential shift.[26] While **2** displays shifted potentials of +0.81 and −2.08 V in acetonitrile vs $\mathrm{Fc^+/Fc}$ as expected, the events remain irreversible. Further, the potentials of **2** clearly lie outside the SOD functional limits, consistent with a deactivating role when/if sulfur oxygenation were to occur in NiSOD. As expected, *p*-nitro blue tetrazolium chloride SOD activity assays[27,28] for **1** and **2** were negative.

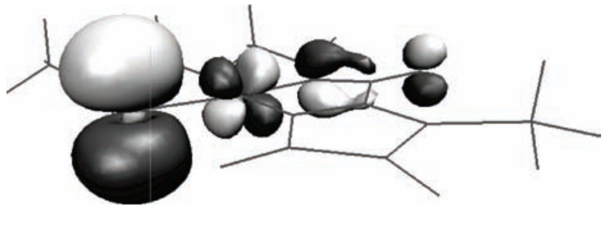


Figure 2. Isosurface plot (isovalue = 0.04) of the HOMO of **1**.

Density functional theory (DFT) calculations on **1** were performed for comparison with our prior study on $(N_2S_2)Ni$ complexes.[14] In that study, sulfur-based O_2 sensitivity was suggested to result from activation of the Ni/S-dominated highest occupied molecular orbital (HOMO) with maximum effects when the S:Ni ratio is near 1:1. Higher S:Ni ratios were observed for O_2-tolerant complexes that required stronger oxidants or electrochemical oxidation for sulfur-based reactivity. The HOMO of **1** (Figure 2) consists of a Ni d/S pπ^* interaction with 49% S p_z and 23% Ni $d_{xz/yz}$ character and a total S:Ni ratio of 2.25:1. The orbital percentages are similar to those of our theoretical amido−amino $(N_2S_2)Ni$ dithiolate (Ni d/S1 p/S2 p = 21%/15%/51%),[14] an O_2-stable amido−amino derivative synthesized and computationally evaluated by Shearer ($\mathrm{Ni_{total}/S_{total}}$ = 33%/50%),[29] and the tethered imidazole $(N_3S)Ni$ complex of Darensbourg et al. (Ni d/S p = 17%/57%).[17] Also consistent with its O_2 stability, a map of the molecular electrostatic potential calculated from Mulliken atomic charges (Figure S4 in the Supporting Information) for **1** reveals a sulfur potential similar to that of our theoretical amido−amino $(N_2S_2)Ni$ dithiolate.

X-ray crystallographic analyses of **1** and **2** reveal $\mathrm{Ni^{II}}$ ions in slightly distorted N_3S square-planar environments with three distinct N-donor types (Figure 3).[30] The Ni−N bond distances in **1** are 1.893(3), 1.869(3), and 1.904(3) Å to the imidazole (N1), carboxamido (N3), and amino (N4) N atoms, respectively. The Ni−N bond distances in **2** are similar, with values of 1.877(3), 1.860(3), and 1.890(3) Å for N1, N3, and N4. The Ni−S bond distance decreases slightly from 2.1810(11) Å in **1** to 2.1119(10) Å, consistent with oxygenation effects observed in related nickel complexes.

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(30) Crystal data for **1**: yellow plate, monoclinic, space group $P2_1/c$, a = 24.166(11) Å, b = 5.302(3) Å, c = 11.363(5) Å, β = 97.407(7)°, V = 1443.9(12) $\mathrm{Å^3}$, D_{calcd} = 1.528 $\mathrm{g/cm^3}$, Z = 4. Data were collected on a Bruker Smart Apex CCD using Mo Kα radiation. For all 3293 unique reflections [R(int) = 0.0624], the final anisotropic full-matrix least-squares refinement on F^2 for 185 variable data converged at R1 = 0.0495 and wR2 = 0.1061 with a GOF of 1.05. CCDC 603908. Crystal data for **2**: light-yellow plate, orthorhombic, space group $P2_12_12_1$, a = 6.0103(9) Å, b = 10.1562(15) Å, c = 23.342(4) Å, V = 1424.8(4) $\mathrm{Å^3}$, D_{calcd} = 1.609 $\mathrm{g/cm^3}$, Z = 4. Data were collected on a Bruker Smart Apex CCD using Mo Kα radiation. For all 3371 unique reflections [R(int) = 0.0347], the final anisotropic full-matrix least-squares refinement on F^2 for 188 variable data converged at R1 = 0.0412 and wR2 = 0.0962 with a GOF of 1.052. CCDC 603909.

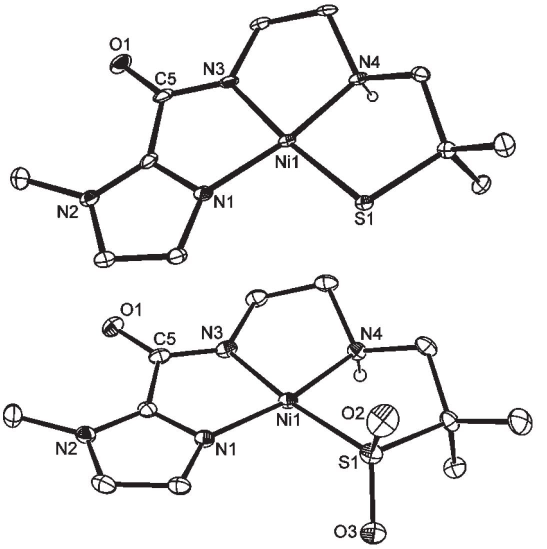

Figure 3. (top) ORTEP representation of **1**. Selected bond lengths [Å] and angles [deg]: Ni1−N1 1.893(3), Ni1−N3 1.869(3), Ni1−N4 1.904(3), Ni1−S1 2.1810(11); N1−Ni1−S1 100.47(9), N1−Ni1−N3 83.77(12), N3−Ni1−N4 85.32(12), N4−Ni1−S1 90.57(9). (bottom) ORTEP representation of **2**. Selected bond lengths [Å] and angles [deg]: Ni1−N1 1.877(3), Ni1−N3 1.860(3), Ni1−N4 1.890(3), Ni1−S1 2.1119(10), S1−O2 1.486(3), S1−O3 1.460(3); N1−Ni1−S1 101.53(9), N1−Ni1−N3 84.34(13), N3−Ni1−N4 85.30(13), N4−Ni1−S1 88.68(9).

The Ni−N/S bond distances of **1** and **2** are similar to those of $\text{NiSOD}_{\text{red}}$.[5,6]

In conclusion, complex **1** incorporates Ni^{II} in a planar, dianionic N_3S chelate with three N-donor types (amino, amido, and imidazole) and a single thiolate. Like the $[(N_3S)\text{Ni}]^+$ complexes reported by Darensbourg et al., **1** contains an in-plane imidazole, in contrast to the axial histidine of NiSOD_{ox}.[17] Recent spectroscopic and theoretical studies by Fiedler et al. concluded that *axial* histidine ligation promotes reversible metal-based oxidation.[22] Our spectroscopic and computational results confirm that *in-plane* imidazole supports sulfur-based oxidation similar to diamino and amido−amino (N_2S_2)Ni complexes.[14,29,31] As reported previously, the nucleophilicity of the sulfur is optimized by raising the energy of the HOMO as indicated by a S:Ni ratio close to unity, leading to complexes that are O_2-sensitive.[14,32] Complexes with higher S:Ni ratios, such as **1**, tend to be resistant to O_2 oxidation, although they display irreversible sulfur-based oxidations electrochemically and sulfur oxygenation upon the addition of strong oxidants including H_2O_2.[14,29] This is consistent with the protection of nickel thiolates, including **1** and NiSOD, from O_2 via "kinetic control".[17,32] Whether this protection extends to H_2O_2 oxidation remains unresolved.

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Supporting Information Available: Experimental procedures, crystallographic details, electronic spectra of **1** and **2**, the IR spectrum of **2**, a map of the molecular electrostatic potential of **1**, the ^{1}H NMR spectrum of **1**, and X-ray structural data in CIF format (CCDC 603908 and 603909). This material is available free of charge via the Internet at http://pubs.acs.org.

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