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# A Square-planar Ni(II) Complex with an Asymmetric $N_2S_2$ Donor Set as a Model for the Active Site of Nickel-containing SOD: Structural Conversion Driven by Addition of a Strong Donor Ligand in the High Oxidation State

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A novel Ni(II) complex with an asymmetric monoamino–monoamidato–dithioether square-planar coordination (**1**) was prepared as a model compound of the active center of NiSOD and characterized by spectroscopic and electrochemical methods. EPR data indicate that the structure of complex **1** in the high oxidation state can be significantly altered upon binding of imidazole, a stronger donor ligand than the original supporting ligand.

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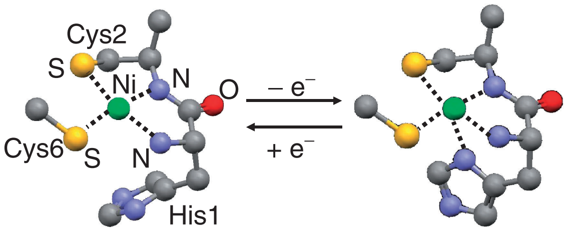
Ni-containing superoxide dismutase (NiSOD) represents a new class of SOD which catalyzes disproportionation of the superoxide radical through the Ni(II) and Ni(III) states, as shown in the following reactions.[1]

$$\mathrm{Ni(II)} + \mathrm{O_2}^{-\bullet} + 2\mathrm{H}^+ \rightarrow \mathrm{Ni(III)} + \mathrm{H_2O_2} \quad (1)$$

$$\mathrm{Ni(III)} + \mathrm{O_2}^{-\bullet} \rightarrow \mathrm{Ni(II)} + \mathrm{O_2} \quad (2)$$

Crystal structures of the active site have been reported for both the Ni(II) and Ni(III) states. In the Ni(II) state, the central metal ion has a square-planar structure with equatorial ligands provided by one amino group of the terminal histidine, one amidato nitrogen of the peptide backbone, and two thiolato sulfurs of cysteine residues.[2] When the Ni(II) center is oxidized to Ni(III), the imidazolyl group of the terminal histidine becomes coordinated at one of the axial positions with a relatively long ligand-to-metal distance of ca. 2.5 Å (Scheme 1).[2] The existence of this structure is also supported by an EPR spectrum typical of a $d_{z^2}$ ground state with a triplet hyperfine splitting in the $g_{\parallel}$ region.[1–3] On the other hand, the EPR spectrum of the H1Q mutant of NiSOD (H1Q-NiSOD), in which the terminal histidine is replaced with glutamate, was also found to exhibit a triplet hyperfine splitting in spite of lacking the histidine imidazole.[3] Consequently, the active site structure of the oxidized NiSOD remains controversial. Some studies using small Ni(II) complexes as a model compound of NiSOD active center have been performed. They have mainly described their structures, spectroscopic characterizations, and electrochemical properties, and they have discussed the proton pathway required in the reducing process of superoxide. However, nothing has been reported concerning the reaction with superoxide, and no solutions have been proposed for the above controversy.[4–8]



**Scheme 1.** Schematic structures of NiSOD active site in the reduced (left) and oxidized (right) states.

In this study, we prepared a Ni(II) complex with a square-planar structure coordinated by an asymmetric amino–amidato–dithioether ligand, $[\mathrm{Ni^{II}(L_{-H})^-}](\mathrm{ClO_4})$, (L = *N*-(2-methylmercapto-2-methylpropanoyl)-*N*′-(2-methylmercapto-2-methylpropyl)-1,2-diaminoethane) (**1**), as an active site model of NiSOD and examined its structural behavior in the Ni(III) state induced by different oxidizing agents and addition of exogenous ligands.

The crystal structure of **1** is shown in Figure 1 together with selected bond lengths and angles.[9] The Ni(II) center of **1** has a square-planar geometry coordinated by $N_2S_2$-donor sets. The bond length of Ni–N1 (amidato nitrogen) (1.845(3) Å) is shorter than that of Ni–N2 (amino nitrogen) (1.909(2) Å) because it is a stronger donor than the amino nitrogen. The bond length of Ni–S1 (2.1772(9) Å) is slightly longer than that of Ni–S2 (2.1692(10) Å). This may be due to the difference in the C–C–N bond angles between the amidato and the amino moieties. These bond lengths are consistent with those of Ni(II) complexes with $N_2S_2$ coordination containing three 5-memberd chelate rings.[4,10–12] Complex **1** has an absorption band at 436 nm ($\varepsilon$/M$^{-1}$ cm$^{-1}$ = 390) in acetone. This band is characteristic of Ni(II)–$N_2S_2$ complexes with square-planar geometry coordinated by an amidato group.[4,10,11] Native NiSOD also exhibits a similar absorption band.[13] The Ni(II) complex **1** produces reversible redox potentials corresponding to the Ni(I)/Ni(II) and Ni(II)/

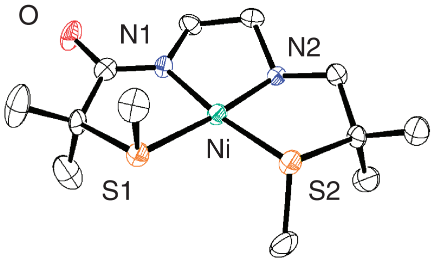


**Figure 1.** ORTEP drawing of the cation moiety of **1** with thermal ellipsoids drawn at 50% probability. The hydrogen atoms and counter anions (perchlorate) are omitted for clarity. Selected bond lengths (Å) and angles (°): Ni–N1 = 1.845(3), Ni–N2 = 1.909(2), Ni–S1 = 2.1772(9), Ni–S2 = 2.1692(10), N1–Ni–S2 = 172.46(9), N2–Ni–S1 = 173.83(9), N1–Ni–S1 = 87.96(8), S1–Ni–S2 = 96.63(3), N2–Ni–S2 = 89.40(8), N1–Ni–N2 = 85.91(11).

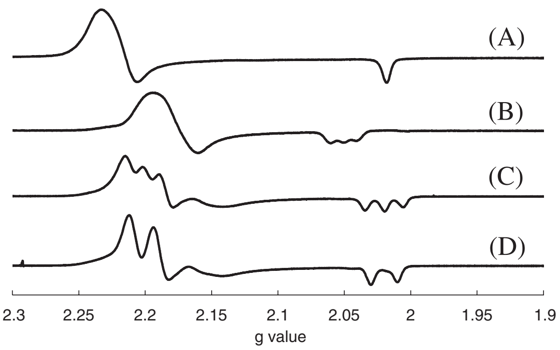


**Figure 2.** EPR spectra observed (A) after oxidation of **1** using $(NH_4)_2[Ce(NO_3)_6]$ (in MeOH), (B) after oxidation using $KO_2$ (in MeOH), (C) by addition of 7.5 equiv of imidazole to resulting product oxidized by $(NH_4)_2[Ce(NO_3)_6]$ (in a 4:1 mixture of acetone and DMF), and (D) by addition of 7.5 equiv of $^{15}N$-labeled imidazole to the resulting product oxidized by $(NH_4)_2[Ce(NO_3)_6]$ (in a 4:1 mixture of acetone and DMF).

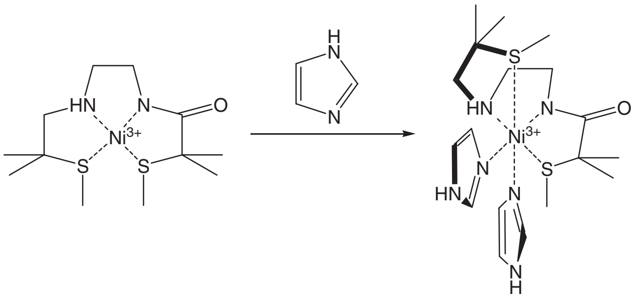


**Figure 3.** Proposed structural change of the oxidized complex of **1** induced by addition of imidazole.

Ni(III) couples at −1.13 and +1.26 V (vs. NHE). The potential of the Ni(II)/Ni(III) couple is much higher than the potential range which is known to be suitable for SOD function (−0.16 to +0.89 V).[14] It seems that the higher redox potential is adopted because the overall charge of the Ni(II) complex **1** is positive, while the complex at the active center of native NiSOD has an overall negative charge.

The oxidation of the Ni(II) complex by ammonium cerium(IV) nitrate ($(NH_4)_2Ce(NO_3)_6$) produces an EPR spectrum which is typical of a low spin $d_{z^2}$ ground state ($g_\perp = 2.22$, $g_\parallel = 2.02$) in MeOH, suggesting that the Ni(II) center of **1** is oxidized to a low spin Ni(III) state (Figure 2A). The larger $g_\perp$ value ($g_\perp = 2.22$) is characteristic for Ni(III) complexes with square-planar structure.[15,16] This structure may have been preferred because of the Jahn–Teller effect. On the other hand, when the Ni(II) complex is oxidized by potassium superoxide ($KO_2$) in the same solvent, the oxidized product exhibits an EPR spectrum characteristic of a $d_{z^2}$ ground state with a triplet hyperfine splitting in the $g_\parallel$ region ($g_\perp = 2.17$, $g_\parallel = 2.05$, $|A_\parallel| = 1.5$ mT, Figure 2B) although no exogenous ligands have been added. This observation clearly indicates that the axial position of the complex is occupied by an amino or amidato nitrogen with $I = 1$ upon addition of the oxidizing agent $KO_2$. The structure of the complex is converted from a square planar to another distinct structure with an amino- or amidato-nitrogen atom occupying the axial position as a result of the oxidation. To obtain an approximation of this converted structure, imidazole was added to the oxidized product of complex **1** as an exogenous ligand. Interestingly, the EPR spectrum obtained by the oxidation of complex **1** by $(NH_4)_2Ce(NO_3)_6$ showed a triplet hyperfine splitting both in the $g_\perp$ and $g_\parallel$ regions ($g_\perp = 2.20$, $|A_\perp| = 1.7$ mT, $g_\parallel = 2.02$, $|A_\parallel| = 2.3$ mT) (Figure 2C). These splittings were changed from triplet to doublet ($g_\perp = 2.20$, $|A_\perp| = 2.5$ mT, $g_\parallel = 2.02$, $|A_\parallel| = 3.5$ mT) when $^{15}N$-labeled imidazole with $I = 1/2$ was used as an exogenous ligand (Figure 2D).

These findings suggest that the two imidazole molecules are coordinated to the Ni(III) center of the complex at the equatorial and axial positions. We previously reported Ni(II) and Ni(III) complexes with an asymmetric $N_3S_2$ ligand containing an amino–amidato–dithioether moiety with a pyridine pendant.[17] In this report, the equatorial plane of the metal center is occupied by amino, amidato, pyridine nitrogen ligands and a thioether sulfur of the amidato group. The remaining thioether sulfur is weakly coordinated at the axial position of the metal center. Based on these facts, we propose that the thioether sulfur which is linked to the amino nitrogen of the oxidized complex **1** can be easily "kicked up" to the axial position of the metal center when a stronger donor is provided, as shown in Figure 3. In addition to this structural change, when $KO_2$ is used as an oxidizing agent, the peroxide anion generated by oxidation of Ni(II) ion of **1** becomes strongly bound to the Ni(III) center at an equatorial position and alters the equatorial plane. As a result of this structural change, the amino nitrogen of the supporting ligand is shifted to the axial position of the Ni(III) center.

As mentioned above, the oxidized H1Q mutant of NiSOD also exhibits triplet hyperfine splitting in the $g_\parallel$ region in the EPR spectrum in spite of the lack of the terminal hystidine.[3] Our Ni(II) complex which has a flexible $N_2S_2$ square-planar structure indicates another possibility which may explain this controversial result. As seen in the crystal structure of native NiSOD, the active center structures of H1Q- and native NiSODs may also have flexibility similar to complex **1**.[2] We, therefore, propose that the thiolate sulfur atom of Cys6 residing on the equatorial plane of the active center of both of H1Q- and native NiSOD can be kicked up to the axial position when a stronger donor such as superoxide or peroxide anion is provided. Especially in the case of H1Q-NiSOD the amine nitrogen may have coordinated at the axial position as a result of this structural change.

In summary, we prepared a new Ni(II) complex with an asymmetric square planar $N_2S_2$ coordination structure as a model compound of NiSOD active site and got the following three aspects. (i) We first succeeded in observation of the oxidation of Ni(II) complex as a model compound of NiSOD active site to the Ni(III) state by the reaction with superoxide. (ii) In the oxidized state of **1**, the sulfur atom in the equatorial plane can be kicked up to the axial position when a strong donor is given as an exogenous ligand. (iii) This structural change gives us an idea about the structure of oxidized H1Q-NiSOD, namely, as the result of this structural change, the coordinating site of amine nitrogen can be moved from equatorial to axial position.

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9 Crystallographic data of **1**: $C_{12}H_{25}ClN_2NiO_5S_2$, fw 435.61, monoclinic, $P2_1/c$, $a = 10.9799(8)$ Å, $b = 8.0567(5)$ Å, $c = 21.4381(16)$ Å, $\beta = 98.494(4)°$, $V = 1875.6(2)$ Å$^3$, $Z = 4$, $D_{\text{calcd}} = 1.542\ \text{g cm}^{-3}$, unique reflns. ($R_{\text{int}}$) 4028 (0.048), $R$ ($I > 2\sigma(I)$)/$R_w$ (all reflections) = 0.0414/0.0777, GOF = 0.885.

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