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Synthesis of an N_3S_2-type Ni(II) Complex Directed to NiSOD Active Site and Its Structural, Electrochemical, and Spectroscopic Properties

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An N_3S_2-type Ni(II) complex (**8**) has been synthesized as a model compound of NiSOD active site, which is coordinated with an amide, an amine, and a pyridyl nitrogens and a thioether sulfur atom in the equatorial positions and with the remaining sulfur weakly bound at the axial position. The UV-vis spectroscopic and electrochemical characterizations of complex **8** showed that the square pyramidal structure was kept in the solution in both the Ni(II) and Ni(III) states. In Ni(III) state, EPR spectrum exhibited a rhombic signal pattern without any hyperfine splittings, indicating no structural change depending on oxidation states. If a protonation to the coordinated thiolate in Cys2 or Cys6 of NiSOD is caused during the oxidative disproportionation process, the thiolate may be kicked up to the axial position because the donor ability of the sulfur has been weakened.
Key words: NiSOD, Model compound, Ni complex and Bioinorganic chemistry

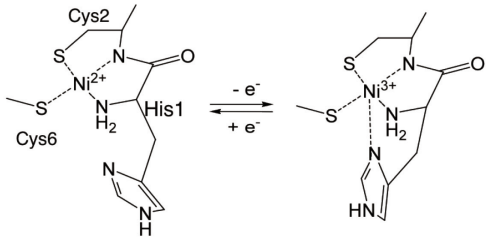
1. INTRODUCTION

Anionic superoxide radical ($O_2^{\cdot-}$) is an unavoidable by-product in aerobic metabolism and it causes serious oxidative damages of biomolecules. To avoid this harmful oxidation, all aerobic organisms have a metalloenzyme called superoxide dismutase (SOD). The SOD catalyzes disproportionation of superoxide radical to hydrogen peroxide (H_2O_2) and dioxygen (O_2) through redox process of the metal ion. Ni-containing superoxide dismutase (NiSOD) was recently discovered, which catalyzes disproportionation of superoxide radical through the Ni(II) and Ni(III) states, as shown in the following reactions[1].

$$Ni(II) + O_2^{\cdot-} + 2H^+ \rightarrow Ni(III) + H_2O_2 \quad (i)$$

$$Ni(III) + O_2^{\cdot-} \rightarrow Ni(II) + O_2 \quad (ii)$$

Crystallographic study has revealed that the enzyme has six active sites, each of which includes either Ni (II) or Ni(III) oxidation state. In the Ni(II) state, the metal ion has a square planar geometry with one amine nitrogen of His1, one deprotonated amide nitrogen in the peptide backbone, and two thiolates from Cys2 and Cys6 residues [2-3]. In the Ni(III) state, on the other hand, it employs a five coordinated square pyramidal structure where an imidazolyl nitrogen at the terminal histidine further interacted to the axial position (Scheme 1) [2-3].



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

In this study, we designed Ni(II) complex with an N_3S_2-type ligand containing an amide, an amine, and a pyridyl nitrogens and two sulfurs, which was characterized by using electrochemical and spectroscopic methods.

2. EXPERIMENT

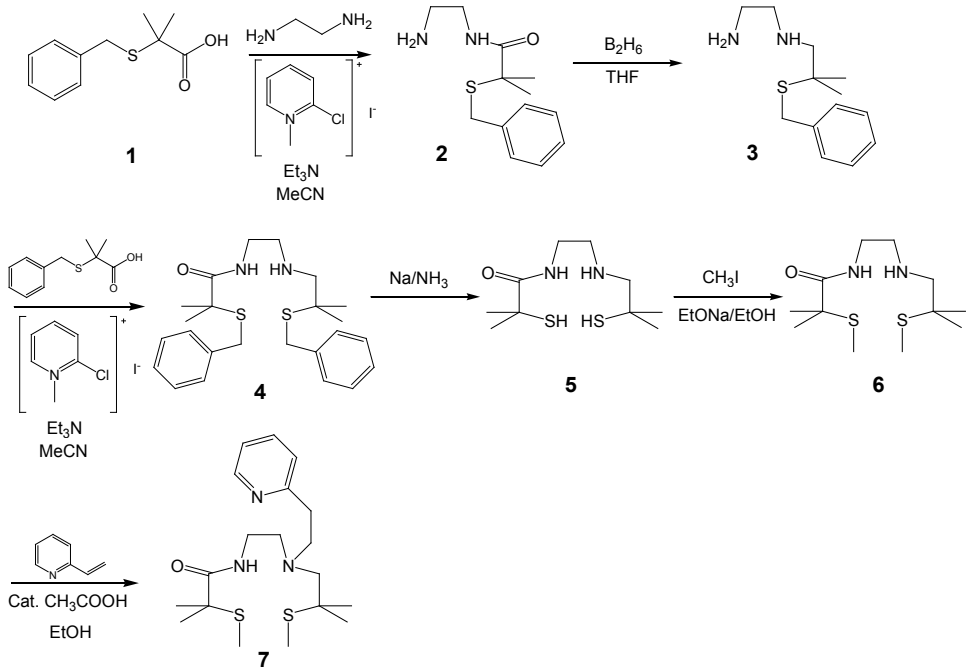
Synthetic scheme of ligand is depicted in Scheme 2.

2.1 Preparation of Ligand

2-Benzylmercapto-2-methylpropanoicacid (**1**) [4] and 2-chrolo-1-methylpyridiniumiodide [5] were prepared according to the methods previously reported. Benzylmercaptane and methyliodide were purchased from Nacalai Tesque INC., and $Ni(ClO_4)_2 \cdot 6H_2O$ was obtained from Sigma-Aldrich Co. All other reagents and solvents from Wako Pure Chemical Industry Co. were used without further purifications. All reagents were the highest grade available.

2.1:1 *N*-(2-Benzylmercapto-2-methylpropanoyl)-1,2-diaminoethane (**2**)

Triethylamine (2.0 g, 19 mmol) and **1** (4 g, 19 mmol) were dissolved in MeCN (100 ml). 2-Chrolo-1-methyl-pyridinium iodide (4.8 g, 19 mmol) was added to the solution and stirred for 1 hr. The solution was dropwised into 1,2-diaminoethane (20 ml) and stirred overnight. The solvent and unreacted 1,2-diaminoethane were removed in *vacuo* to afford an oily orange residue. The oil was dissolved to an alkaline aqueous solution at pH 12 and was extracted three times with AcOEt (50 ml). The AcOEt layer was washed with saturated NaCl and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure to afford **2** as clear oil. (Yield 3.1 g, 63 %). δ($CDCl_3$, 300 MHz); 1.56 (s, 6H), 2.78 (t, 2H), 3.20 (q, 2H), 3.76 (s, 2H), 7.29 (m, 5+1H).



Scheme 2. Synthetic scheme of ligand

2.1:2 *N*-(2-Benzylmercapto-2-methylpropyl)-1,2-diaminoethane (**3**)

Etheral trifluoroboran solution (12.8 g, 90 mmol) was added to the THF solution (50 ml) of $NaBH_4$ (1.7 g, 45 mmol) and stirred for 10 min. The solution was added to THF solution (150 ml) of **2** (3.1 g, 12.3 mmol). The resultant mixed solution was refluxed for 20 hr. A little amount of MeOH was added to the solution to decompose the remaining B_2H_6. After addition of H_2O (100 ml) to the solution, the organic solvent was evaporated off. The resulting aqueous solution was extracted three times with AcOEt (50 ml) at pH 12. The AcOEt layer was dried with anhydrous sodium sulfate (Na_2SO_4) after being treated by saturated NaCl solution. AcOEt was removed under reduced pressure to afford **3** as clear oil. (Yield 2.0 g, 70 %). δ($CDCl_3$, 300 MHz); 1.34 (s, 6H), 1.86 (br, 2H), 2.51 (s, 2H), 2.54 (q, 2H), 2.73 (t, 2H), 3.70 (s, 2H), 7.32 (m, 5H).

2.1:3 *N*-(2-Benzylmercapto-2-methylpropanoyl)-*N'*-(2-benzylmercapto-2-methylpropyl)-1,2-diaminoethane (**4**)

Triethylamine (0.88 g, 8.35 mmol) and **1** (1.77 g, 8.35 mmol) were dissolved in MeCN (100 ml). 2-Chrolo-1-methylpyridinium iodide (2.1 g, 8.35 mmol) was added to the solution and stirred for 1 hr. Compound **3** in MeCN (50 ml) was introduced into the solution, followed by being stirred overnight. The solvent was removed under reduced pressure to afford an oily yellow material. The oil was extracted three times with Et_2O (50 ml) at pH 12. The organic layer was washed with saturated NaCl and dried with Na_2SO_4. The solvent was removed under reduced pressure to afford **4** as clear oil. The oil was purified by column chromatography with $CHCl_3$. (Yield 2.6 g, 69 %). δ($CDCl_3$, 300 MHz); 1.32 (s, 6H), 1.53 (s, 6H), 2.47 (s, 2H), 2.54 (t, 2H), 3.17 (q, 2H), 3.67 (s, 2H), 3.73 (s, 2H), 7.21-7.35 (m, 10H), 7.37 (br, 1H).

2.1:4 *N*-(2-Mercapto-2-methylpropanoyl)-*N'*-(2-mercapto-2-methylpropyl)-1,2-diaminoethane (**5**)

Compound **4** (2.6 g, 5.8 mmol) was dissolved into dry THF (5 ml). Na (1 g, 43 mmol) was added to the solution, after liquid ammonia (100 ml) was introduced into the solution at -78 ºC. After stirring for 30 min., NH_4Cl was added until the blue color of the solution was completely disappeared. The ammonia was removed at r. t. and H_2O (50 ml) was added to the residue under Ar atmosphere. After the solution was washed with Et_2O (50 ml) at pH 12, the resulting solution was extracted three times with AcOEt (50 ml) at pH 8. The AcOEt layer was dried with Na_2SO_4 after treating with saturated NaCl solution. The organic solvent was removed to afford **5** as a clear oil. (Yield 1.25 g, 83 %). δ($CDCl_3$, 300 MHz); 1.38 (s, 6H), 1.60 (s, 6H), 2.62 (s, 2H), 2.85 (t, 2H), 3.35 (q, 2H), 7.46 (br, 1H).

2.1:5 *N*-(2-Methylmercapto-2-methylpropanoyl)-*N'*-(2-methylmercapto-2-methylpropyl)-1,2-diaminoethane (**6**)

To an EtOH solution (100 ml) of **5** was added Na (0.24 g, 10.3 mmol). Methyliodide (1.47 g, 10.4 mmol) was dropwised to the solution and the resultant mixed solution was stirred for 3 hrs. at 40 ºC. After removing EtOH under reduced pressure, the residue was dissolved in H_2O (100 ml). The solution was extracted three times with Et_2O (50 ml) at pH 12. The ether was removed under reduced pressure to afford **6** as oil. The crude oil was purified by column chromatography with $CHCl_3$. (Yield 1.25 g, 86 %). δ($CDCl_3$, 300 MHz); 1.29 (s, 6H), 1.50 (s, 6H), 1.98 (s, 3H), 2.05 (s, 3H), 2.56 (s, 2H), 2.80 (t, 2H), 3.36 (q, 2H), 7.45 (br, 1H).

2.1:6 *N*-(Ethylpyridyl)-*N*-(2-mercapto-2-methylpropanoyl)-*N'*-(2-mercapto-2-methylpropyl)-1,2-diaminoethane (**7**)

2-Vinylpyridine (0.378 g, 3.60 mmol) and **6** (0.5 g, 1.80 mmol) were dissolved into EtOH (100 ml). The solution was refluxed for 8 days with catalytic amount of CH_3COOH. After removing EtOH by evaporation, the residue was extracted with CH_2Cl_2 at pH 12. The CH_2Cl_2 layer was dried with Na_2SO_4 after treating with a saturated NaCl solution. The organic solvent was removed by evaporation to afford an oily brown crude material. The oil was purified by passing through a silica gel column with $CHCl_3$. (Yield 50 mg, 6.8 %). δ($CDCl_3$, 300 MHz); 1.27 (s, 6H), 1.51 (s, 6H), 2.02 (s, 3H), 2.04 (s, 3H), 2.61 (s, 2H), 2.82 (t, 2H), 2.96 (m, 2H), 3.35 (q, 2H), 7.12 (t, 1H), 7.16 (d, 1H), 7.60 (t, 1H), 8.53 (d, 1H).

2.2 Preparation of complex **8**

Sodium methoxide (7.07 mg, 0.13 mmol) and **7** (50 mg, 0.12 mmol) were dissolved into MeOH (5 ml). MeOH (5 ml) solution of $NiCl_2 \cdot 6H_2O$ (31.1 mg, 0.13 mmol) was dropwised to the solution, which was stirred for 3 hrs. After removing the solvent in *vacuo*, the resultant solution was dissolved into MeCN (2 ml) and filtered. Sodium tetraphenylborate (44.8 mg, 0.13 mmol) and H_2O (5 ml) were added to the MeCN solution to afford **8** as a pale brown precipitate. The precipitate was recrystallized from a vapor diffusion method of diethyl ether to the acetone solution.

2.3 Measurements of samples

Electronic absorption spectra were taken on a JASCO U-best V-550 spectrometer at r. t.. ^{1}H-NMR spectra were taken on a Varian Gemini-300 NMR spectrometer. X-band EPR spectra of frozen solutions were recorded at 77 K by using a JEOL JES-RE 1X ESR spectrometer. Cyclic voltammetric measurements were performed using ALS/CH Instruments Electrochemical Analyzer Model 600A. Glassy-carbon, Ag/Ag^+, and Pt-wire electrode were used as working, reference, and counter electrodes, respectively. All measurements were performed in acetone solution containing 0.1 M $(n\text{-Bu})_4NBF_4$ electrolyte at r. t. under Ar atmosphere.

2.4 X-ray crystallography

A single crystal of complex **8** was mounted on a glass fiber. X-ray measurement was made on a Rigaku Mercury CCD area detector with graphite monochromated Mo-Kα radiation. The data were collected at a temperature of -100 ± 1°C to a maximum 2θ value of 25.0°. A total of 1200 oscillation images were collected. The structure was solved by direct methods [6] and expanded using Fourier techniques [7]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

3. RESULTS AND DISCUSSION

3.1 Characterization of **8**

The crystal structure of **8** was determined by an X-ray analysis, as shown in Fig.1 [8]. The Ni(II) complex has a square pyramidal structure (τ = 0.06) [9] with one deprotonated amide, one amine, and one pyridine nitrogens and one thioether sulfur in the equatorial positions and the other thioether sulfur weakly interacted at the axial position (2.787(3) Å). Interestingly, the thioether sulfur was kicked up at the apical position. This may have been caused by the weaker coordination of relatively flexible thioether compared with the pyridyl nitrogen, which is often found in Cu(II) or Ni(II) complexes with thioether and pyridine as the donor atoms [10-11]. This flexible coordination of sulfur atom is similar to the coordination situation of Cys6 in NiSOD active site, although there is a difference between thioether of complex **8** and Cys6 thiolate in NiSOD active site.

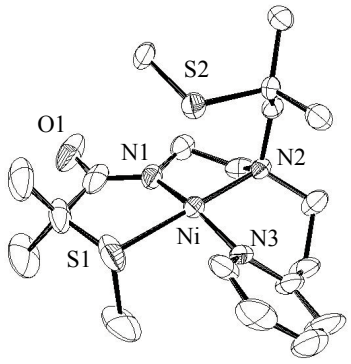


Fig.1 ORTEP drawing of an anion part of **8** with thermal ellipsoids drawn at the 30 % probability. The hydrogen atoms and counter anion (BPh_4) are omitted for clarity. Selected bond distances (Å) and angles (°): Ni-N1 = 1.870(10), Ni-N2 = 1.969(8), Ni-N3 = 1.929(9) Ni-S1 = 2.192(4), Ni-S2 = 2.787(3), N1-Ni-N2 = 86.2(4), N2-Ni-N3 = 94.9(3), N1-Ni-S1 = 87.8(3), N3-Ni-S1 = 90.2(3), N1-Ni-N3 = 169.5(4), N2-Ni-S1 = 173.0(2), N1-Ni-S2 = 99.2(3), N2-Ni-S2 = 84.3(2), N3-Ni-S2 = 91.2(3), S1-Ni-S2 = 100.47(14).

The complex **8** showed an absorption band at 472 nm (ε / M^{-1} cm^{-1}: 250) in acetone. The absorption band is characteristic for Ni(II) complexes with a square planar [12-17] or a square pyramidal [18] geometry. In order to confirm the structure in solution, ^{1}H-NMR spectrum of **8** was also measured. It exhibited broadened signals in paramagnetic region (11.3-21.0 ppm) in acetone-d_6, indicating that the central Ni(II) ion is in a high spin state with a square pyramidal coordination geometry.

A quasi-reversible redox wave corresponding to $Ni^{2+/3+}$ of **8** was observed at 0.99 V (vs. NHE) in acetone (ΔE = 94 mV), indicating that the structure around metal center does not change upon the redox process. The potential for $Ni^{2+/3+}$ is much higher than those of negatively-charged [Ni^{II}-N_2S_2] complexes with a square planar structure, $[Ni(emi)]^{2-}$ (-0.50 V) [12], and $[Ni(beaam)]^-$ (-0.04 V) [13], but relatively similar to those of neutral complexes, [Ni(bmmp-dmed)] (1.04 (E_{pa}) V) [14] and [Ni(ema)·$(Me)_2$] (1.24 V) [15]. Thus, the net charge of the complex and donor ability of ligating atom influence on redox potentials of Ni(II) complexes.

Oxidation of Ni(II) complex **8** by cerium ammonium nitrate ($[Ce(NH_4)_2(NO_3)_6]$) gave a rhombic EPR signal typical for dz^2 ground state (g = 2.20, 2.13, 2.01) as shown in Fig.2, indicating that the Ni(II) center of **8** was oxidized to a low spin Ni(III) species with an asymmetrical coordination environment. A singlet peak in g_z region implies that all nitrogen atoms

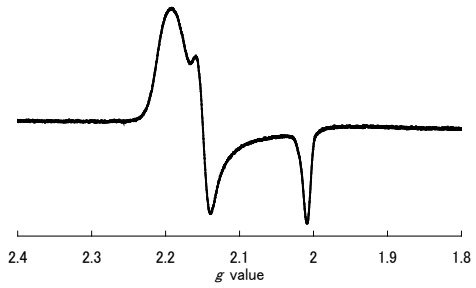


Fig.2 Frozen solution EPR spectrum of the oxidized form of complex **8.** The sample was prepared in acetone solution (5 mM) by oxidizing at 195 K using 1 eq. of $[Ce(NH_4)_2(NO_3)_6]$ as oxidizing agent.

coordinate to the equatorial plane of the Ni(III) center. This result suggests that there is no essential structural change at the Ni center in 3+ oxidation state. The *g*-values were relatively lower than other Ni(III) complexes with a square planar structure [12,16,19-20]. This fact indicates that the d-d energy separation for **8** is smaller because smaller splitting in the d-orbitals theoretically gives larger *g*-values [21]. It is therefore concluded that **8** holds a square pyramidal structure even after oxidation.

3.2 Biological implications

We prepared the Ni(II) complex of N_3S_2-type ligand with a square pyramidal structure as the active site model of NiSOD. The UV-vis spectrum and X-ray structure revealed that the square pyramidal structure of **8** was kept in both the solution and solid states. Electrochemical and EPR spectroscopic studies showed that the structure of **8** does not essentially change in the redox process. In **8**, the thioether sulfur has been kicked up at the apical position and weakly coordinated to the metal ion. The Cys6 sulfur in NiSOD active site may be similar to the coordination situation around the NiSOD active center, because the Cys6 residue is coordinated to the metal center as a quite unique flexible ligand. These results made us assumed the biological implication as described below.

In NiSOD active center in the oxidized Ni(III) state, a histidyl imidazole interacted at the axial site of metal center [2-3], which was also supported by EPR study as follows [1-2,22]. The oxidized NiSOD gave an EPR spectrum typical for dz^2ground state and showed a triplet hyperfine splitting at the $g_z$signal, which is typical for a low spin Ni(III) species with a square planar or a square pyramidal structure. This finding indicates that the axial coordination is generally thought to be an imidazole of His1 residue. However, H1Q mutant of NiSOD (H1Q-NiSOD) also gave a triplet hyperfine splitting in spite of lacking the histidyl imidazole [22], suggesting that the coordination of other nitrogen atoms rather than hystidyl one is plausible in the active state during the superoxide disproportionation. In the oxidative disproportionation process, the protonation to the coordinated thiolate in Cys2 or Cys6 of NiSOD is proposed [23-24]. Here, we speculate that the thiolate of Cys6 may be kicked up to the axial position because of its weakened donor ability.

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[8] Crystallographic data for **8**: $C_{43}H_{52}BN_3NiOS_2$, F.W.: 760.53, triclinic, *P*-1, a = 11.488(6) Å, b = 13.165(7) Å, c = 13.751(8) Å, α = 89.013(13)°, β = 87.479(10)°, γ = 82.146(13)°, V = 2058.0(20) Å^3, Z = 2, ρ_{calcd} = 1.227 g/cm^3, unique refls. (R_{int}) 9420 (0.043), R/R_w ($I > 2\sigma$ (I)) = 0.0925/0.1370, GOF = 1.000.
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