A Novel Nickel(II) Complex with 1,4-Dithia-8,11-diazacyclotetradecane Derivative as a Structural Model of the Nickel Site in [FeNi]-Hydrogenases

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A new Ni(II) complex with 8-(2-pyridylmethyl)-1,4-dithia-8,11-diazacyclotetradecane ligand (P[14]aneN₂S₂), [NiP[14]aneN₂S₂(H₂O)](ClO₄)₂ (**1**), was prepared as a structural model of [FeNi] hydrogenase active site. The X-ray crystal structure analysis of the complex, in which the coordinated water molecule was replaced by Br⁻, revealed an octahedral geometry coordinated with P[14]aneN₂S₂ and Br⁻. The cyclic voltammograms of complex **1** gave only a reversible wave of Ni^{2+/+} couple at $E_{1/2} = -920$ mV vs. SCE in MeOH and two reversible waves of Ni^{2+/+} and Ni^{3+/2+} couples at $E_{1/2} = -970$ and 1350 mV vs. SCE in MeCN, respectively, indicating that complex **1** can take a wide range of oxidation states of nickel ion. The reduction of complex **1** with NaBH₄ in MeOH at -20 °C gave the Ni(I) complex with a hydride ion at the axial position, which was demonstrated by ESR spectroscopy.

Key words: [FeNi] hydrogenase, Nickel(II) complex, ESR, Hydride

1. INTRODUCTION

[FeNi] Hydrogenases [1] are metalloenzymes that catalyze the reversible two-electron oxidation of dihydrogen ($\text{H}_2 \rightleftharpoons 2\text{H}^+ + 2\text{e}^-$) and are involved in the production and consumption of H₂ by various microorganisms, in which the Ni site is considered to play as the active center for the uptake and oxidation of H₂. Recently the X-ray crystal structures of [FeNi] hydrogenases isolated from *Desulfovibrio gigas* [2a] and *Desulfovibrio vulgaris* Miyazaki [2b] revealed that the Ni site is penta-coordinated with four thiolate sulfurs of cystein residues and one S/O atom. X-ray absorption spectroscopy of the [FeNi] hydrogenase from the purple photosynthetic bacterium *Thiocapsa roseopersicina* indicated that the active nickel center exists most probably in either trigonal bipyramidal or six-coordinate with 2 ± 1 S(Cl)-donor ligands at 2.23 ± 0.03 Å and 3 ± 1 N(O)-donor ligands at 2.00 ± 0.06 Å.[3] ESR study of the [FeNi] hydrogenase indicated the spectral behaviors suggesting that the Ni site goes through three different redox states of Ni(I), Ni(II), and Ni(III).[1c] [FeNi] hydrogenase isolated from *Desulfovibrio gigas* under air, which is in fully oxidized and enzymatically inactive states, displayed two kinds of rhombic ESR signals; Ni-A form (an unready state) ($g=2.31, 2.23$, and 2.02) and Ni-B form (a ready state) ($g=2.33, 2.16$, and

2.02).[1] The oxidized forms under H₂ atmosphere were ESR silent. Additional exposure of H₂ to the hydrogenase resulted in appearance of another rhombic ESR signals of Ni-C form ($g=2.19, 2.14$, and 2.01) which is enzymatically active.[1] Extensive incubation of the enzyme under H₂ led to formation of the fully reduced form (R). The Ni-C form has been deduced to be in the Ni(III) or Ni(I) state coordinated with H⁻ or H₂ on the basis of the results of ESR and ENDOR experiments.[1,4] This Ni-C form has also been supported to be in the Ni(I) state,[1,5] because it reversibly reacts with CO.

Recently, with the aim of making clear some problems such as the oxidation states of the nickel center, coordination of H⁻/H₂, redox properties of nickel ion, and so on, many structural and/or functional model studies have been carried out by use of the nickel complexes with sulfur atoms as coordination sites for hydrogen.[6,7,8] We are also interested in constructing the model complex having these coordination features, and we newly synthesized the Ni(II) complex of 8-(2-pyridylmethyl)-1,4-dithia-8,11-diazacyclotetradecane (P[14]aneN₂S₂; Scheme I).

Here, we will describe the structural characterization and electrochemical property and also the reaction of the complex with some reductants useful for understanding the function

of the hydrogenases.

Scheme 1. Ligand P[14]aneN₂S₂

2. EXPERIMENTAL

2.1 Materials and Measurements.

Reagents and solvents employed were of the highest grade available. All solvents for spectroscopies were purified by further distillation before use. Other chemicals were used without further purification.

Electronic absorption spectra were taken on a JASCO Ubest-35 spectrophotometer. The samples for spectral measurements were prepared in MeOH and MeCN. Cyclic voltammetric measurements were performed using a Bioanalytical Systems (BAS) CV-1B instrument equipped with a Graphtec X-Y WX2400 chart recorder. A 3-mm diameter glassy-carbon working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a Pt-wire counter electrode were used in a glass cell having a working compartment. All measurements were made at 25 °C under an argon atmosphere in MeOH and MeCN with tetra(n-butyl)ammonium tetrafluoroborate (0.1 M) as a supporting electrolyte at a scan rate of 100 mV/s. X-band ESR spectra of frozen solutions were recorded at 77K by using a JEOL RE-1X ESR spectrometer. ESR samples were prepared in MeOH solution in quartz tubes, and the reactions were performed in the temperature range -20 ~ 0 °C under an argon atmosphere. NaBH₄ and Na₂S₂O₄ were employed as a reductant. The reductions of complex were carried out by the two methods; (i) addition of 1.2 equiv of Na₂S₂O₄ in 0.1 mL of H₂O and (ii) addition of 1.2 equiv of NaBH₄ in 0.1 mL of ethanol. Elemental analyses were performed on a Leco CHN-900 analyzer.

2.2 Preparations of P[14]aneN₂S₂ Ligand.

To a CH₂Cl₂ (100 mL) solution containing [14]aneN₂S₂ [9] (0.53 g, 2.5 mmol) and triethylamine (0.30 g, 3 mmol) was added a CH₂Cl₂ (100 mL) solution of 2-(bromomethyl)pyridine (0.52 g, 3 mmol) from a dropping funnel at a rate of about 1 mL/min. The mixed solution was stirred, whereby the reaction was followed by TLC (silicagel, CHCl₃-MeOH (7:1)). After completing the reaction, the solvent was evaporated off, which were separated by column chromatography on silicagel with CHCl₃-MeOH

(10:1). Yield: 0.16 g (0.5 mmol), 20 %.

2.3 Preparation of [NiP[14]aneN₂S₂(H₂O)]-(ClO₄)₂ (**1**).

Complex **1** was prepared by mixing an MeOH solution (5 mL) of P[14]aneN₂S₂ (0.16 g, 0.5mmol) and an MeOH solution (5 mL) of Ni(ClO₄)₂·6H₂O (0.18 mg, 0.5mmol). Purple plate-like crystals of [NiP[14]aneN₂S₂(H₂O)]-(ClO₄)₂ were obtained by slow diffusion of hexane into the MeOH solution of the reaction product. Yield 0.17 g (0.3 mmol), 66 %. Anal. Calc. for C₁₆H₂₉Cl₂NiN₃O₉S₂: C, 31.96; H, 4.86; N, 6.99. Found: C, 31.73 H, 4.77; N, 6.81.

Crystal used for X-ray analysis was obtained from the reaction of **1** with KBr in MeOH solution.

2.4 X-ray Structure Analyses of Complex **1**.

A single crystal of complex **1** was mounted on a glass capillary. The diffraction data were collected with graphite-monochromated Mo-Kα radiation on an Enraf-Nonius CAD4-EXPRESS four-circle diffractometer at room temperature with the ω-2θ scan technique. Crystallographic data and experimental details are described in the reference [10].

The structure was solved by a combination of the direct method and the Fourier technique, and they were anisotropically refined for non-hydrogen atoms by full-matrix least-squares calculation. Refinements were continued until all shifts were smaller than one-tenth of the standard deviations of the parameters involved. Atomic scattering factors and anomalous dispersion terms were taken from International Tables for X-Ray Crystallography.[11] Empirical absorption correction was applied after the full isotropic refinement of non-hydrogen atoms. Hydrogen atoms were refined with fixed isotropic temperature coefficients; they were located at the calculated positions. The structure analysis was carried out on an SGI IRIS Indigo XS-24 workstation by using the teXsan program [12] by using the SDP-MoLEN program.[13]

3. RESULTS AND DISCUSSION

3.1 Solution and Crystal Structures.

The complex **1** gave the absorption bands characteristic of a six-coordinate octahedral one [14] in the visible region in both MeOH and MeCN; 340 (sh 70), 550 (10), and 860 (10) in MeOH and 335 (sh 90), 525 (20) and 820 nm (20 M⁻¹cm⁻¹) in MeCN, respectively.

The X-ray structure of complex **1** was analyzed using a single crystal of [NiP[14]aneN₂S₂Br]ClO₄ that the water molecule was replaced by Br⁻ because it was fortunately obtained as a single crystal. As depicted in Fig. 1, the structure of the [NiP[14]aneN₂S₂Br]⁺ cation part in the crystal was a distorted octahedron with one amino nitrogen (Ni - N(1) = 2.091(5) Å), one pyridyl nitrogen (Ni - N(3) = 2.081(5) Å), and two sulfurs

Figure 1. Crystal structure of a cation part of **1**. Hydrogen atoms were omitted for clarity.

(Ni - S(1) = 2.437(2), Ni - S(2) = 2.389(2) Å) of P[14]aneN₂S₂ in the equatorial plane and with one amino nitrogen (Ni - N(2) = 2.107(5) Å) and one Br⁻ (Ni - Br = 2.580(1) Å) at the axial positions. The Ni-Br distance, 2.580 Å, is somewhat longer than typical values for Ni-Br lengths (2.536-2.572 Å), [14] although the other Ni-donor atom bond lengths are normal.

3.2 Electrochemistry and Chemical Reduction.

Cyclic voltammogram for complex **1** was measured in MeOH (Fig. 2(a)) and MeCN (Fig. 2(b)). That in MeOH exhibited only one

Figure 2. Cyclic voltammograms of **1** in (a) MeOH and (b) MeCN

reversible wave assignable to the Ni^{2+/+} couple at $E_{1/2}$ = -920 vs. SCE, and that in MeCN gave two reversible waves of Ni^{2+/+} and Ni^{3+/2+} couples at $E_{1/2}$ = -970 and 1350 mV vs. SCE, respectively.

Addition of NaBH₄ to MeOH solution of complex **1** at room temperature resulted in a drastic color change. After a few hours of the addition, the color was returned to that of the starting Ni(II) complex again. The absorption spectrum, as followed at -20 °C under Ar, gave

new two absorption bands at 350 nm (sh) and 510 nm (ϵ 130), which are assigned to the charge-transfer bands from ligand to metal and the d-d bands of Ni(I) complexes generated, respectively.

X-band ESR spectra of the Ni(I) complex in MeOH solution, which was reduced by Na₂S₂O₄, exhibited a axial pattern with g = 2.01, 2.11, and 2.21, indicating that a single unpaired electron lies on the dx^2-y^2 orbital of the nickel atom. Interestingly, that of the Ni(I) complex prepared by NaBH₄ exhibited a rhombic pattern with g = 2.23, 2.17, 2.04, showing the dz^2 ground state (Fig. 3). Furthermore, the signal of g = 2.04 was split

Figure 3. ESR spectrum of **1** reduced by NaBH₄ in MeOH.

in doublet (A = 50 G), suggesting that a nuclear spin of I = 1/2 is located on the dz^2 orbital of the Ni(I) center. [15] The fact that this hyperfine splitting was observed not in the use of Na₂S₂O₄ but in NaBH₄ indicates that hydride anion is located on the dz^2 orbital. Similar large doublet nuclear hyperfine splitting of Ni(I)-H⁻ (A = 100 G) was observed in HI-Ni(CO)₄ system. [16]

Regrettably, the Ni(III) complex was not generated for complex **1**, although the oxidation reactions with [Ce(NO₂)₃](NH₄)₂ and (Et₄N)₃[Fe(CN)₆] were applied. The result indicates that it is difficult to chemically oxidize the Ni(II) complex **1** to the Ni(III) species.

3.3 Biological Implications.

The ESR spectral feature of the product complex obtained by the reduction of complex **1** with NaBH₄ is remarkably similar to that of the Ni-C Form in *Thiocapsa roseopersicina* hydrogenase. The ESR spectral evidence on the formation of hydrido-Ni(I) species opens a way to reveal the reaction mechanism at the active Ni site of the hydrogenases. Retention by bonding of a hydrogen atom on the central metal atom of the complexes **1** indicates that the Ni atom is surrounded with the N₂S₂N(py)-pentacoordinate ligand of restricted backbone structure and controlled electronic structure of the central metal atom, or more specifically [NiP[14]aneN₂S₂X] (X: coordination site for hydride), may be profitable for artificial construction of an active Ni site of the enzyme. Further investigation related to these ligands is in progress.

4. CONCLUSION

In order to construct the model complex of active sites in [FeNi]-hydrogenases that catalyze the reversible oxidation of dihydrogen, [1] the Ni(II) complex **1** was synthesized and the solution and crystal structures and electrochemical properties were investigated. The complex has been characterized by electronic absorption spectroscopy, cyclic voltammetry (CV), and X-ray structure analysis. Absorption spectra of the complex **1** demonstrated mainly to be 6-coordinate in solutions. The solution structure of all Ni(II) complex corresponded well to the crystal structure. The redox potential of **1** exhibited considerably lower values and suggested that they can easily be controlled by appropriate selection of donor ligands. Reactions of the Ni(II) complex with reductants exhibited very interesting ESR spectral behaviors: In complex **1**, the reaction with NaBH₄ showed unique spectra which are characteristic for a rhombic pattern with a remarkably large hyperfine splitting at g_z ($A = 50$ G); this spectral pattern is quite similar to that of the Ni-C species in *Thiocapsa roseopersicina* hydrogenase. Such a spectral feature of the large splitting was not observed in the reaction with Na₂S₂O₄. The above findings may indicate that the ESR spectrum detects the Ni(I) ion with an axially-bound hydride ion. We believe that the results obtained in this study will provide important information for further studies on the structure-function relationship of hydrogenases.

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