Effect of an Asymmetrically Arranged Equatorial Chelate Ring in an Active Site of Nitrile Hydratase

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A Co^{III} complex with a novel N_2S_2-type ligand, asymmetrically arranged with 6-5-5 chelate ring, was prepared as an active site model of nitrile hydratase and was found to show a higher stability constant in the axial ligation of *t*-butylisocyanide compared with the previously reported symmetric analog.

Nitrile hydratases (NHases), which are classified to Co^{III}- and Fe^{III}-type ones, are enzymes that hydrate a nitrile compound to the corresponding amide. Both active metal centers are octahedrally surrounded by two amide nitrogen atoms from the peptide backbone and two oxidized cystein sulfur atoms (Cys–SO and Cys–SO_2) in the equatorial plane and a cystein sulfur atom and H_2O (Co-type) or NO (Fe-type) at the axial positions.[1–4] These coordination environments around the metal center include three interesting structural features. One is the existence of three different kinds of sulfur atoms, cystein (S), sulfenyl (SO), and sulfinyl sulfurs (SO_2). The second is the coordination of amide nitrogen atoms which are linked to the neighboring carbonyl oxygen atoms though the hydrogen-bonding networks with some water molecules in the protein. The third is the existence of asymmetrically arranged equatorial plane composed of the 6-5-5 chelate rings. The five- and six-membered chelate rings are coordinated with sulfenyl and sulfinyl groups, respectively.

The former two features have been studied using some Co^{III} and Fe^{III} model complexes with N_2S_3-,[3] N_3S_2-,[4,5] or N_2S_2-type[6–12] ligands containing amide or imine nitrogen atoms. However, the third one was remained unsolved. The equatorial chelate rings studied hitherto were symmetrically arranged with 5-6-5[6,7,10] or 6-5-6.[8,9] We previously reported the influence of an electrophilic interaction between solvent molecules and carbonyl oxygen atoms on the Lewis acidity of the central metal ion by using N_2S_2-type Co^{III} complex ($(PPh_4)[Co(\mathbf{L1})]$ (**1**); $H_4\mathbf{L1}$ = *N*,*N′*-bis(2-mercapto-2-methylpropionyl)-1,3-diaminopropane) whose chelate rings were also symmetrically arranged.[10] Here, we report the effect of the asymmetric arrangement of the chelate rings using a Co^{III} complex **2** with a new "asymmetric" N_2S_2-type ligand, (3-mercapto-3-methyl)butanoyl-aminoacetoyl-[*N*-(2-mercapto-2-methyl)propyl]amine ($H_4\mathbf{L2}$), which is similar to the active center of NHase in the structural features of not only the coordination atoms but also the arrangement of the chelate rings.

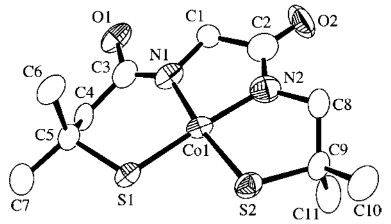

Figure 1. An ORTEP view of the $[Co(\mathbf{L2})]^-$ anion in **2**, showing 50% probability ellipsoids. Selected bond lengths (Å) and angles (°): Co1–N1, 1.890(4); Co1–N2, 1.903(4); Co1–S1, 2.145(1); Co1–S2, 2.141(1); N1–Co1–N2, 85.1(2); S1–Co1–S2, 87.81(5); S1–Co1–N1, 100.5(1); S1–Co1–N2, 174.0(4); S2–Co1–N1, 171.4(4); S2–Co1–N2, 86.7(4).

The Co^{III} complex with **L2**, $(Me_4N)[Co(\mathbf{L2})]$ (**2**), was prepared by the same method as $(PPh_4)[Co(\mathbf{L1})]$[10] except for the use of tetramethylammonium chloride in place of tetraphenylphosphonium chloride. The crude product obtained from an aqueous solution was recrystallized from CH_2Cl_2/toluene to afford single crystals suitable for X-ray analysis. An ORTEP view of the complex anion, $[Co(\mathbf{L2})]^-$, is shown in Figure 1. The Co^{III} ion is surrounded with two amide nitrogen and two sulfur atoms to form a slightly distorted square-planar structure with asymmetrically arranged chelate rings (6-5-5), which is just the same as that of NHase as expected. The averaged bond lengths of Co–S and Co–N_{amide} are typical for a square-planar Co^{III} complex (Co–S: 2.13–2.14 Å, Co–N_{amide}: 1.86–1.92 Å).[10–12] The bond angles, S1–Co1–N1 (100.5(1)°) and N1–Co1–N2 (85.1(2)°), however, are largely different from other Co^{III} complexes reported hitherto (S1–Co1–N1 (87.8–89.4°) and N1–Co1–N2 (93.2–98.0°)),[10–12] which is predominantly caused by the asymmetric configuration of the 6-5-5 chelate rings. Consequently, the sulfur atom in a five-membered chelate ring coordinates to the metal center with a deviation from the plane formed by the metal ion and two five-membered ones. This is indicative of the difference in the electrostatic influence on the metal center and on the coordinated atoms.

Cyclic voltammograms of **2** were measured in several solvents. The voltammograms showed a quasi-reversible redox wave assignable to the Co^{III}/Co^{II} couple in the potential range of -1.6 to -0.8 V vs. Fc/Fc^+. Plots of acceptor numbers (ANs) of solvents against the redox potentials, in which AN is an index of its electrophilicity, are shown in Figure 2. The linear relationship between the redox potentials and ANs was also found in the case of **1**.[10] The redox potentials of **2** are about 150–200 mV higher than those of **1**. This difference clearly indicates that Co^{III} for **2** receives weaker electrostatic perturbations from the coordinating donor atoms than **1**, although the coordination atoms and their positions in the equatorial plane are almost the same each other. Thus, the Lewis acidity of the Co^{III} center for **2** was expected to be higher than **1**. So, the coordination equilibrium of **2** with *t*-BuNC was also spectrophotometrically examined in order to compare the equilibrium constants of **1** and **2** in CH_2Cl_2. The coordination behavior of **2** is quite similar to that of **1**,[10] so the equilibrium is defined with the following eq 1.

$$[\mathrm{Co}(\mathbf{L2})]^- + 2t\text{-BuNC} \overset{K}{\rightleftharpoons} [\mathrm{Co}(\mathbf{L2})(t\text{-BuNC})_2]^- \qquad (1)$$

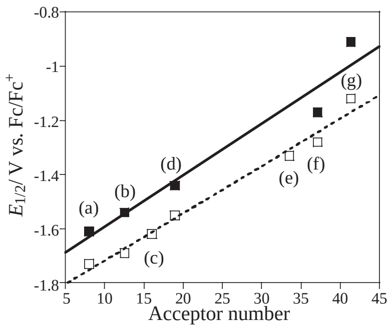

Figure 2. The redox potentials $E_{1/2}$ for complex **1** (□) and **2** (■) were plotted against ANs of organic solvents; (a) THF, (b) acetone, (c) DMF, (d) MeCN, (e) 2-PrOH, (f) EtOH, (g) MeOH.

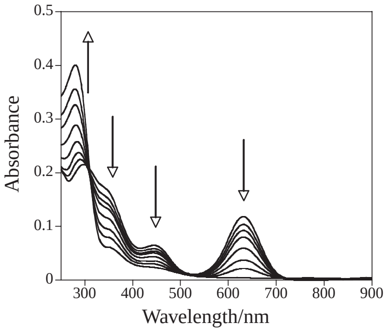

Figure 3. UV–vis spectral changes of complex **2** in EtCN. Measurements were performed by decreasing the temperature from −20 to −55 °C.

The evaluated constants (log K) are 3.13 for **1**[10] and 4.40 for **2**, respectively. The constant for **2** is one order larger in magnitude than **1**, indicating that the Lewis acidity of **2** is higher than that of **1**.

Coordination behavior of **2** with nitrile was examined using propionitrile (EtCN). Complex **2** exhibited a characteristic spectrum for the square-planar structure in EtCN, indicating that the solvent nitrile does not coordinate to **2**. Lowering the temperature down to −55 °C induced the spectral change with an isosbestic point at 312 nm (Figure 3). The spectrum at −55 °C is in good agreement with that of $[\mathrm{Co}(\mathbf{L2})(t\text{-BuNC})_2]^-$ (Figure S1),[13] suggesting the generation of $[\mathrm{Co}(\mathbf{L2})(\mathrm{EtCN})_2]^-$. While, as the temperature was increased to room temperature, the absorption spectrum of **2** recovered. This result indicates that the solvent EtCN can reversibly coordinate to the metal center. When complex **1** was dissolved in EtCN, generation of EtCN-bound $\mathrm{Co^{III}}$ complex was detected even at −55 °C although it was only 30% (Figure S2). Thus, we can consider that the asymmetric coordination environment as seen in NHase intrinsically induces the coordination of the substrate nitrile to the axial vacant site, because the other $\mathrm{Co^{III}}$–N(amide)$_2$S$_2$-type model complexes[11] with "symmetrically" arranged chelate rings in this study did not show any coordination of them to the central metal except for **1**.

Mulliken population analyses for **1** and **2** were carried out using their crystal structures by DFT method. The net charges on the $\mathrm{Co^{III}}$, S, and N atoms are listed in Figure S3.[13] The positive net charge on the $\mathrm{Co^{III}}$ ion for **2** is somewhat larger than **1**, indicating the difference in Lewis acidity. This is consistent with the results of the coordination equilibrium of t-BuNC and electrochemical studies. As to the sulfur and nitrogen atoms in **2**, the negative electron density tends to increase except for that on the N2 atom as compared with **1**. The electron density of N2 atom indeed decreases, although it was not completely superior. By paying attention to the coordinating atoms of **2**, the electron density on S1 atom was significantly larger than that on S2; the former has a six-membered chelate ring and the latter a five-membered one. This result makes us expect that S2 atom included in the six-membered chelate ring is likely to be oxidized easier than S1 in five-membered one.

In conclusion, complex **2** with a N(amide)$_2$S$_2$-type ligand, which has "asymmetrically" arranged chelate rings (6-5-5) similar to the active site structure of NHase, promoted a ligation to the axial position. The DFT calculation revealed that the electron density on the S atom in six-membered chelate ring relatively increased as compared with another one. Thus, we can conclude that the oxygenation in the post-translational modification of NHase starts from the S in the six-membered ring.

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