Coordination of a Water Molecule to a Square-pyramidal N_2S_3-type Co(III) Complex Directed to an Active Site of Nitrile Hydratase

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A novel distorted square-pyramidal N_2S_3-type Co(III) complex with a vacant site was synthesized as an active center model of nitrile hydratases (NHases) and characterized by UV–vis and ^1H NMR spectroscopic and X-ray analyses. The electronic absorption spectra were characteristic of a five-coordinate structure in organic solvents such as acetonitrile, while the complex in an aqueous solution gave different spectrum characteristic of a six-coordinate octahedron. These results suggest that the coordination of a water molecule is a key for the hydration of nitrile compound in the NHases.

Nitrile hydratases (NHases) are known as an enzyme that hydrolyzes a nitrile compound to the corresponding amide. The NHases are divided into two classes according to whether they contain a Co(III) or a Fe(III) ion.[1] X-ray structural analyses were recently performed for both the enzymes,[2,3] both of which revealed that the completely same coordination environments are provided around their mononuclear metal centers: They are comprised of two amide nitrogens and three sulfurs coordinated to the metal ion, in which two sulfurs are oxidized to a sulfinate and a sulfenate each other. A water molecule interacts with the metal ion with 2.58 Å for the Co(III)-type enzyme. Such a unique coordination environment makes an understanding of the hydration mechanism difficult.

Several investigations for model complexes using pentadentate ligands that have coordination environments similar to the active site of NHases have been performed.[4–6] Kinetics for releasing of the sixth monodentate ligand (N_3^-) from the octahedral coordination has been studied on the iron(III) complexes using N_3S_2-type ligands with two azomethine nitrogen, amine nitrogen and two sulfur atoms,[4] and it has been described that the steric strain caused by changing the number of the chelate rings increases the kinetic lability of the monodentate ligand. Iron(III) complexes with N_2S_3-type ligands derived from two aliphatic tertiary amines as nitrogen donors have also been synthesized and characterized.[5] The octahedral Co(III) complexes consisted of one monodentate CN^- ion and an N_3S_2-type ligand including two amide and one pyridine nitrogens have also been studied, and it has been reported that the ligand exchange rate of monodentate CN^- by a water molecule in an aqueous solution is much faster than that expected for usual Co(III) complex.[6] Thus, the pentadentate ligands have often been used as the model compounds of the NHase. However, the donor sets of the ligands lack the similarity of those for the NHase active site. Therefore, we designed and synthesized a novel N_2S_3-type pentadentate ligand, bis[*N*-(2-mercapto-2-methylpropioyl)aminopropyl]sulfide (H_2**L1**), which provides more suitable donating atoms of two amide nitrogens, two thiolate sulfurs, and a thioether sulfur.

A mononuclear Co(III) complex with **L1** was prepared by mixing an equimolar amount of $[Co(NH_3)_5Cl]Cl_2$ and fully-deprotonated species (H_{-2}**L1**) of **L1** treated with NaH in DMF under Ar atmosphere. Exposure of the solution to the air caused an autooxidation of the thiolate sulfurs to form the corresponding sulfinate species. Addition of the ethylacetate into an acetonitrile solution containing the sulfinate species and PPh_4Cl afforded a crude compound ($PPh_4[Co(H_{-2}$**L1-O$_4$**$)]$; **1**).

Recrystallization of the crude material from an acetone/ethylacetate mixed solution gave a single crystal suitable for an X-ray analysis, which revealed that it was a 5-coordinate mononuclear Co(III) complex with two sulfinates, two amide nitrogens, and a thioether sulfur (Figure 1). Taking into consideration that it usually adopts an octahedral conformation because of the large ligand field stabilization energy,[7] this configuration is quite unique. Bond lengths of Co1–N(amide) fall within the usual range (1.91–1.94 Å), while the lengths of Co1–S(sulfinate) are somewhat shorter than those observed previously for the octahedral Co(III) complexes (2.11–2.19 Å).[6,8–11] This shortening must be caused by the coordinative unsaturation around the Co(III) ion. The bond length of Co1–S1 is, on the other hand, by 0.07 Å longer than those reported hitherto (2.21–2.25 Å),[7] which is probably due to a strong trans influence of a sulfinate. The τ value of **1**, which has often been used for an estimation of the structural distortion for a 5-coordinate complex,[12] is 0.37, which indicates that the complex has a distorted square-pyramidal structure with a large vacant site trans to the sulfinate S2. Strong trans influence by the two sulfinates may be allowed to form the vacant site through suitable arrangement of their configurations to minimize the influence.

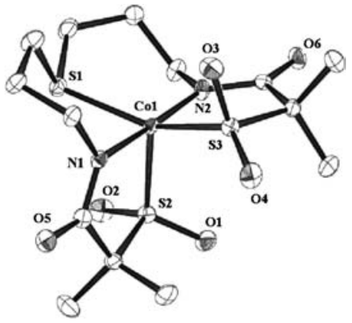

Figure 1. An ortep drawing of $[Co(H_{-2}$**L1-O$_4$**$)]^-$ anion. Selected bond lengths (Å) and angles (deg): Co1–N1 1.931(3), Co1–N2 1.907(3), Co1–S1 2.322(1), Co1–S2 2.107(1), Co1–S3 2.101(1), N1–Co1–N2 177.0(1), S1–Co1–S2 99.13(5), S1–Co1–S3 154.88(5), S2–Co1–S3 105.58(5), S1–Co1–N1 83.6(1), S1–Co1–N2 96.0(1), S2–Co1–N1 86.8(1), S2–Co1–N2 96.2(1), S3–Co1–N1 93.2(1), S3–Co1–N2 85.9(1).

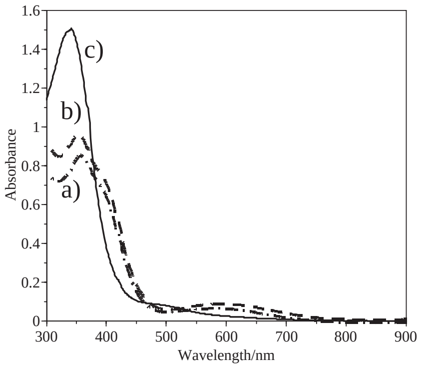

Figure 2. Electronic absorption spectra of **1**. In a) chloroform, b) acetonitrile, c) aqueous solution; [complex] = 1.0×10^{-4} mol dm^{-3}.

^{1}H NMR spectra of **1** in both of CDCl$_3$ and D$_2$O were observed within a diamagnetic region (0–10 ppm), indicating that the spin state of **1** is an S = 0. The previously reported Co(III) complex of a N$_3$S$_2$-type ligand with two azomethine nitrogen, an aliphatic amine nitrogen, and two thiolate sulfur atoms showed a distorted square pyramidal structure ($\tau = 0.48$) with diamagnetic character when one thiolate was oxidized to sulfinate.[11]

Electronic absorption spectra of **1** in chloroform and acetonitrile are shown in Figures 2a and 2b, respectively. Both the spectra gave the same feature with peak maxima at around 590 nm ($\varepsilon \approx 700\,\mathrm{M^{-1}cm^{-1}}$) and 355 nm ($\varepsilon \approx 8000\,\mathrm{M^{-1}cm^{-1}}$), indicating that the acetonitrile molecule, which acts as a substrate of hydration, does not coordinate to the vacant site of the complex. Similar spectra were also observed in organic solvents such as methanol, DMF, and acetone. On the other hand, the spectrum of **1** in an aqueous solution demonstrated significantly different one (Figure 2c; 456 nm (ε 940 M^{-1}cm^{-1}), 336 nm (ε 10200 M^{-1}cm^{-1})), which is characteristic for octahedral Co(III) complexes with sulfur and nitrogen atoms.[7,9,13] The complex **1** indicated an interesting temperature dependence in the presence of water; decrease of the temperature caused the spectral change from the square-pyramidal feature to the octahedral one with three isosbestic points at 370, 452, and 520 nm in methanol/water (9:1) solution (Figure 3). This result indicates that a water molecule coordinates to the vacant site of **1** to form an octahedral geometry. Interestingly, the spectral feature of complex **1** with a water is similar to that of a Co-type NHase showing an absorption band at 410 nm and a broad band near 450–800 nm,[14] suggesting that the Co-type NHase, as proposed in the literature,[6] contains a water molecule.

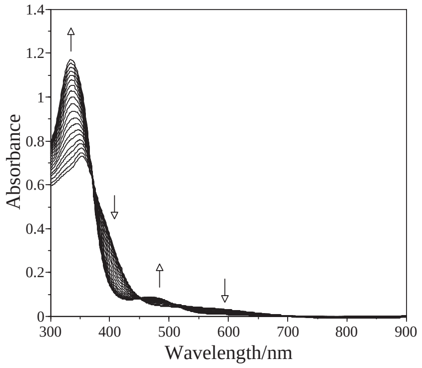

Figure 3. Temperature dependence of absorption spectra for **1** in methanol/water (9:1) solution. Measurement was performed by decreasing the temperature from 0 to −95 °C with intervals of 5 °C. [complex] = 1.0×10^{-4} mol dm^{-3}.

The coordination of water molecule in an aqueous solution was confirmed also from an ESI–MS spectral measurement. An aqueous solution of **1** exhibited a signal with an isotopic feature at $m/z = 489.1$ in negative mode, which is in good agreement with the spectral simulation of [Co(H$_{-2}$**L1-O$_4$**)(H$_2$O)]$^-$.

In conclusion, a novel square-pyramidal Co(III) complex **1** with N$_2$S$_3$-type ligand composed of two amide nitrogens, two sulfinate sulfurs, and a thiolate sulfur was synthesized and characterized as an active center of the Co-type NHase. The finding that a water molecule specifically coordinated to the vacant site of the complex **1** in an aqueous solution is interesting in relation to the hydration process in the NHase enzyme.

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