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Article

Co(III) Complexes with N₂(SO)₂-Type Equatorial Planar Ligands Similar to the Active Center of Nitrile Hydratase: Role of the Sulfenate Group in the Enzymatic Reaction

Takuma Yano,[†] Yuko Wasada-Tsutsui,[‡] Hidekazu Arii,[†] Syuhei Yamaguchi,[†] Yasuhiro Funahashi,[†] Tomohiro Ozawa,*,[†] and Hideki Masuda*,[†]

Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan, and Graduate School of Natural Sciences, Nagoya City University, Mizuho-ku, Nagoya 467-8501, Japan

In order to gain an understanding of the role of the sulfenyl group of nitrile hydratase (NHase), a new Co(III) complex with a sulfenyl-type ligand ($L_{C=O}:N_2(SO)_2$), Na[Co^{III}($L_{C=O}:N_2(SO)_2$)(tBuNC)₂] (**2**), was synthesized. The compound includes two amide groups, two sulfenate sulfurs in the equatorial plane, and two tBuNC molecules in the axial positions. Characterization of the compound was performed by UV–vis spectroscopic, IR spectral, thermogravimetric (TG), and X-ray structure analytical methods. The results are discussed in the context of Co(III) complexes containing the corresponding sulfur-type ($L_{C=O}:N_2S_2$) (**1**) and sulfinyl-type ligands ($L_{C=O}:N_2(SO_2)_2$) (**3**). Complex **2** crystallized with the formula Na[Co^{III}($L_{C=O}:N_2(SO)_2$)(tBuNC)₂]·urea·2H₂O·0.5EtOH. The X-ray structure revealed that the Co(III) complex has an octahedral geometry with Co–S = av. 2.221 Å, Co–N = av. 1.998 Å, and Co–C = av. 1.87 Å. The sulfenyl oxygen and amidate carbonyl oxygen are linked to urea, water, EtOH, and Na⁺ and participate in a hydrogen-bond and an electrostatic interaction. IR and TG measurements demonstrated that the coordination strength of tBuNC to the Co atom increases as follows: **1** < **2** < **3**. Complex **2** has almost the same stability as **3** in all solutions tested, although **1** exhibits a release of axial ligands in nonaqueous solutions. DFT calculations for **1**, **2**, and **3** demonstrated that Milliken atomic charges of the Co(III) centers are +1.466, +1.536, and +1.542, respectively, indicating that the extent of oxidation of the sulfur atoms increases the Lewis acidity of the Co(III) centers. Interestingly, the solution-state IR spectrum of **2** exhibits a solvent-dependent S–O stretching frequency. The frequency decreases with an increase in the electrophilicity (acceptor number) of the solvent. This solvent dependence was not observed for **3**, which has a sulfinate (SO_2) group, suggesting that the sulfenyl oxygen atom has nucleophilic character and promotes strong binding of the tBuNC molecule to lower the reaction barrier. These findings may suggest that the sulfenate oxygen in native NHase acts as a base (proton acceptor) and contributes to the activation of a water molecule and/or nitrile molecule.

Introduction

In recent years, the natural enzymes of microorganisms have been investigated as catalysts for industrial synthetic processes. Nitrile hydratase (NHase) catalyzes the hydration of nitrile compounds to the corresponding amides.[1–3] This enzyme has been used in the first example of an application of an enzyme catalyst for industrial production of acrylamide from acrylonitrile.[4] Although this industrial application represents a successful attempt to harness the catalytic power of a metalloenzymes, the detailed reaction mechanism has not yet been clarified.

The active center common to members of the NHase family contains a Co(III)[5–7] or an Fe(III)[8–10] ion which is

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* To whom correspondence should be addressed. E-mail: masuda.hideki@nitech.ac.jp. Phone: +81-52-735-5228. Fax: +81-52-735-5209.
† Nagoya Institute of Technology.
‡ Nagoya City University.

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coordinated by two oxygenated cysteine sulfurs (Cys-SO and Cys-SO$_2$) and two amidate nitrogens in an equatorial plane. A cysteine sulfur atom occupies one axial site, while a water molecule occupies the sixth site for Co-type[7] NHases. In the Fe-type NHases, an NO molecule occupies the sixth site.[10] Coordination of an amidate nitrogen atom is quite unique in metalloenzymes. Furthermore, coordination of oxygenated cysteine residues of the sulfenate and sulfinate groups can be unpredictable. The sulfenic and sulfinic acids interact with arginine residues, forming a reactive cavity at the interface of the α and β subunits. For example, in the Fe-type NHase from *Rhodococcus N-771*, αCys114-SO and αCys-SO$_2$ are hydrogen bonded with βArg141 and βArg56.[10] Another role of these ligands may be to alter the electronic character of the metal iron center.[11] Interestingly, a recent study using NHase reconstituted without oxygenated sulfurs demonstrated that atmospheric oxygen promotes oxidation of sulfur atoms in a process that rescues the disabled enzymatic reaction.[12] Since hydrolysis of nitriles has not been observed in NHase reconstituted with non-oxygenated sulfur atoms, it has been concluded that the posttranslational modification of the active site of NHase is one of the essential factors in the catalytic reaction. Additionally, further oxidation of the sulfenate to sulfinate caused an inactivation of the enzymatic reaction. This suggests that the sulfenate is an important factor for maintaining the reactivity and that sulfenate has a different role than sulfinate.[13]

Some Co(III) and Fe(III) complexes have been synthesized and characterized as structural/functional models of Co- and Fe-type NHases for the purpose of understanding the unique equatorial coordination feature of the active center of NHase.[14−24] The compound [Co^{III}(N$_2$S$_2$)]NEt$_4$, which has square-planar geometry, was first reported by Artaud et al.[25] The Co(III) center has a spin state of $S = 1$ which is rarely observed in Co(III) complexes. Introduction of CN$^−$ and NO molecules to the axial site was also successfully performed.[25] Also reported was a Co(III) complex with two sulfinated sulfurs, (Et$_4$N)$_2$[Co{N$_2$(SO$_2$)$_2$}(CN)(H$_2$O)], which promotes hydrolysis of acetonitrile to acetamide.[26] Chottard and co-workers also prepared Na[Co^{III}(L-*N$_2$SOSO*)(*t*BuNC)$_2$] and (Me$_4$N)[Co^{III}(L-*N$_2$SO$_2$SO$_2$*)(*t*BuNC)$_2$].[24,27] The former complex hydrolyzed acetonitrile to acetamide, but the latter did not. The conclusion was that the sulfenate group is essential for catalysis of the nitrile hydration reaction.[27]

We reported previously that the Lewis acidity of the central metal ion was controlled by an electrophilic interaction between ligand carbonyl oxygens and solvent using the N$_2$S$_2$-type Co(III) complex, PPh$_4$[Co^{III}(L$_{C=O}$:N$_2$S$_2$)(*t*BuNC)$_2$] (**1**), and the N$_2$(SO$_2$)$_2$-type Co(III) complex, PPh$_4$[Co^{III}(L$_{C=O}$:N$_2$-(SO$_2$)$_2$)(*t*BuNC)$_2$] (**3**).[28] The Lewis acidity, however, was not affected by the interaction between the solvents and the sulfinyl group. In this paper, we focus on the role of the sulfenate group in native NHase and introduce two sulfenate groups to the N$_2$S$_2$-type Co(III) complex to prepare PPh$_4$-[Co^{III}(L$_{C=O}$:N$_2$(SO)$_2$)(*t*BuNC)$_2$] (**2**). Complex **2** was characterized by X-ray structural analyses, electronic absorption spectral measurements, infrared spectral measurements, thermal analyses, and theoretical calculations. In order to clarify the role of the coordinated sulfenate group, we discuss features of complex **2** in the context of the features of **1** and **3**.

Experimental Section

Reagents and Methods. All manipulations were performed using Schlenk techniques under argon or in a glovebox under an argon atmosphere. Reagents used in this study were purchased as reagent grade from Wako Pure Chemical Industry Inc. or Tokyo Kasei Kogyo Inc. and used without further purification. Reagent-grade solvents were purchased from Wako Pure Chemical Industry Inc. and Kanto Kagaku Inc. and used after double distillation.

Preparations. Synthesis of Na[Co^{III}(L:N$_2$(SO)$_2$)(*t*BuNC)$_2$] (2). Na[Co^{III}(L$_{C=O}$:N$_2$S$_2$)] (**1′**), which is a planar metal complex without any axial ligand for complex **1** and prepared according to a previous report[27] with some modifications, was employed as a starting material. To a DMF (1 mL) solution of complex **1′** (134.5 mg, 0.378 mmol) was dissolved a large excess amount of *t*BuNC (601.6 μL, 5.29 mmol). The green solution turned to reddish orange and was then stirred under a nitrogen atmosphere at −15 °C for 10 min. To this solution was added urea−hydroperoxide (417.5 mg,

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Table 1. Crystallographic Data and Experimental Details for Na[Co(L$_{C=O}$:N$_2$(SO)$_2$)(tBuNC)$_2$]·urea·2H$_2$O·EtOH (**2**)

empirical formula	C$_{45}$H$_{88}$Co$_2$O$_{13}$N$_{12}$Na$_2$S$_4$
fw	1297.35
cryst syst	orthorhombic
space group	*Pbca* (No. 61)
a [Å]	18.6246(9)
b [Å]	24.2869(7)
c [Å]	33.3280(9)
V [A^3]	15075.4
Z	8
D_{calcd} [g cm^{-3}]	1.143
$F(000)$	5488.00
μ [cm^{-1}]	6.16
λ [Å]	0.710 70
T [K]	173
no. of reflns measd	120 024
no. of reflns used [$I > 2\sigma(I_0)$]	6935
$R_1{}^a$/$R_w{}^b$	0.073/0.230
GOF	1.25

^{a} $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$. ^{b} $R_w = [\sum w(|F_o| - |F_o|)^2/\sum w|F_o|^2]^{1/2}$; $w = 4F_o{}^2/\sigma^2(F_o)$.

4.54 mmol). After further stirring the solution for 1 h, acetone (100 mL) was added and a reddish orange precipitate was obtained (71 mg, 0.112 mmol), which was then dried under vacuum. Diffusion of diethyl ether into the ethanol solution containing the precipitate afforded **2** as red crystals. The elemental analysis data was different from the formula obtained by X-ray analysis. This is because elemental analysis was carried out after vacuum treatment.

Anal. Calcd for C$_{23}$H$_{45}$N$_6$S$_2$O$_{6.5}$NaCo (Na[Co^{III}(L$_{C=O}$:N$_2$(SO)$_2$)-(tBuNC)$_2$]·urea·H$_2$O·0.5EtOH): C, 42.13; H, 6.92; N, 12.82. Found: C, 41.95; H, 6.78; N, 13.15. ^{1}H NMR (300 MHz; δ ppm from TMS in DMSO-d_6): 1.10 (m, 12H, $-$CH$_3$), 1.34 and 1.40 (2s, 18H, tBu), 3.16 (m, 6H, $-$CH$_2$), 5.42 (s, 4H, urea). Electronic absorption spectrum in ethanol (λ_{max}, nm (ϵ M^{-1} cm^{-1})): 294 (7000), 381 (8000), 450 (sh).

X-ray Crystal Structure Analysis. A single crystal of **2** suitable for X-ray diffraction was mounted on a glass fiber. The diffraction data were collected using the oscillation technique with a Rigaku Mercury diffractometer using graphite-monochromated Mo Kα radiation at -100 °C. Crystal data and experimental details are listed in Table 1. The crystal structures were solved by a combination of direct methods and Fourier techniques. Non-hydrogen atoms were anisotropically refined by full matrix least-squares calculations. Hydrogen atoms were included but not refined. 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 the *International Tables for X-ray Crystallography IV*.[29] All calculations were carried out using a Japan SGI workstation computer using the teXsan crystallographic software package.[30]

Other Physical Measurements. Elemental analyses were performed using a Perkin-Elmer 2400II CHNS/O full-automatic analyzer. All mass spectra were acquired using an LCT mass spectrometer equipped with an ion spray interface (Micromass Limited, Manchester, U.K.). Instrument settings, data acquisition, and data processing were controlled by a computer using the Windows NT operating system. Samples were introduced using a single syringe pump (KD Scientific Inc.) fitted with Hamilton syringes (Hamilton Co., Reno, NE). The samples employed for all spectral measurements were prepared in MeCN or MeOH. ^{1}H NMR

spectra were recorded on a Varian Gemini-300 FT-NMR instrument. Electronic absorption spectra were measured on a JASCO V-550 or V-570 spectrophotometer in the wavelength range of 900$-$280 nm. Match paired quartz cells with 10 mm lengths were used. Infrared spectral measurements were carried out using a JASCO FT/IR 410 spectrophotometer. Solid samples were prepared under a pressure of 5 ton/cm^2 with KBr powder. A CaF$_2$ cell with a path length of 0.025 mm was used for the solution sample. Differential spectra between the samples and the corresponding solvents were adopted to verify the spectral features. Thermogravimetric analysis (TA) measurements were recorded on a Rigaku TAS 300 system with a speed of 5°/min.

Theoretical Calculations. Electronic structural calculations were performed on the basis of the crystal structures of **1**$-$**3**. Atomic charges were computed by carrying out Milliken population analyses using RHF density matrices calculated with minimal basis sets. Contraction of the minimal basis sets were (5333/5333*/5),[31] (533/53),[32] (53/5),[33] and (5)[34] for Co, S, the first-row, and hydrogen atoms, respectively. We also performed second-order perturbation theory analyses of the Fock matrix in natural bond orbital basis to investigate the donor$-$acceptor interaction between Co(III) and ligand.[35] In this analysis Becke's three-parameter hybrid functional using the Lee$-$Yang$-$Parr correlation functional (B3LYP)[36] was applied. The 6-31G(d) basis set[37] was used for all atoms, except that the 6-311G basis set was used for Co.[38] For the basis sets of cyanide carbon, nitrogen, and oxygen atoms diffuse functions (6-31+G(d)) were added.[39] All calculations were carried out with the Gaussian 03 package[40] using the Fujitsu PRIMEPOWER HP2500 instrument of the Nagoya University Information Technology Center.

Results and Discussion

Preparation and Stability of Complex 2. Complex **2** was prepared from the reaction of complex **1**′ with urea$-$

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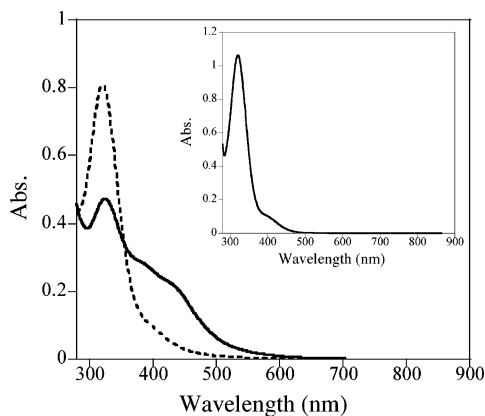
Figure 1. UV−vis spectra of complex **2** in DMF after (a) 2 h standing at ambient temperature (solid line) and (b) 2 h heating at 50 °C (broken line). [complex] = 50 μM. Inset represents a spectrum of complex **3** in DMF.

Figure 2. Plots of S=$O_{sulfenyl}$ (■) and S=$O_{sulfinyl}$ (○) stretching vibration values for complexes **2** and **3** vs ANs of organic solvents: (a) acetone, (b) MeCN, (c) CHCl$_3$, (d) 2-PrOH, (e) EtOH, (f) MeOH.

hydrogen peroxide in the presence of a large excess amount of *t*BuNC in DMF. The complex was very stable in the solid-state even in air, although the SO group of **2** was gradually oxidized to SO$_2$ by atmospheric oxygen in DMF as described below. Complex **2** exhibited red and reddish-orange colors in methanol and DMF, respectively, in contrast to the orange color of complex **3** in both solvents. Complex **2** was apparently affected by solvents. These findings indicate that different electronic structures exist in different solutions. Interestingly, the electronic absorption spectrum of complex **2** exhibits quite different behavior in DMF than in MeOH. In DMF, **2** has a drastic spectral change at 50 °C (Figure 1), although it is unchanged at room temperature even after 2 h. The resultant spectrum in DMF at 50 °C was essentially identical to that of complex **3** in DMF, indicating that the sulfenate group of **2** is oxidized to sulfinate. In contrast, an analogous spectral change was not observed in MeOH even at 50 °C. This solvent effect may be explained in terms of the interaction between the sulfenate oxygen and solvent molecules, as discussed further below in the crystal structure section. The MeOH molecule stabilizes the sulfenate group of **2**, while DMF does not. This behavior may also correlate with the acceptor numbers (ANs) of the solvents, which provide an index of the electrophilic character of the solvent.[41−45] Protic solvents like MeOH (41.3), which has a large AN value, provide stability to the complex, while aprotic solvents such as DMF with an AN value of 16 destabilize the complex. Thus, we can conclude that the attractive interactions of the sulfenate oxygen atoms with the outer-sphere solvent molecules mainly contribute to stabilization of the sulfenate group through electrostatic interactions.

Previously, we reported that **1** exhibits a release of the axial ligand *t*BuNC in nonaqueous solutions, in contrast to a higher stability in **3**. Interestingly, **2** also exhibits essentially
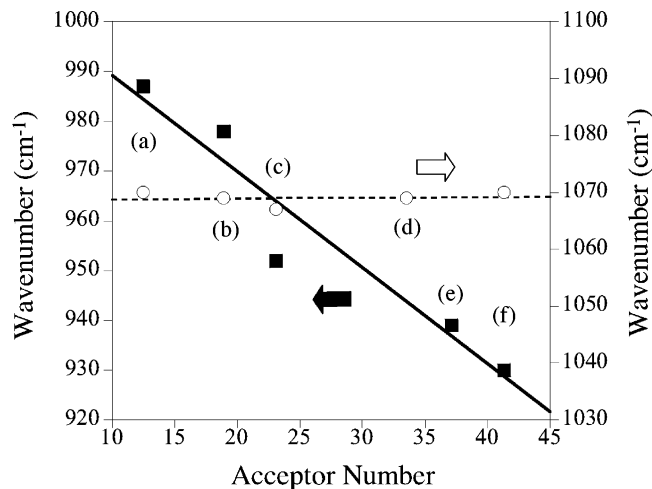
the same high stability as **3** in all solutions used. These results demonstrate that **2** and **3** prefer a six-coordinate geometry over a four-coordinate geometry, while **1** prefers a four-coordinate geometry to a six-coordinate geometry. This was interpreted as follows: the sulfenyl and sulfinyl[46] groups which have electron-withdrawing oxygen atoms raise the Lewis acidity of Co ion to promote tight binding of *t*BuNC.

Solution-State IR Spectra of 2. As pointed out in the above section, complex **2** exhibits a remarkable solvent effect while **3** does not. This is consistent with the observation that the S=O stretching vibrations of **3** do not exhibit a solvent effect. We therefore concluded that the sulfinate oxygen does not participate in a nucleophilic interaction.[28] Here, we performed the infrared spectral measurement of complex **2** in several solvents to quantitatively estimate the interaction with solvent molecules by monitoring the S=O stretching frequency. The solution-state IR spectra of complex **2** were measured using PPh$_4$-**2** in place of **2** with Na$^+$ as a countercation because it is less soluble in all solvents employed.

The frequencies assigned to the S=O stretching vibration were detected in the range of 930−990 cm^{-1}: 987 cm^{-1} in acetone (AN = 12.0), 978 cm^{-1} in MeCN (AN = 18.9), 952 cm^{-1} in CHCl$_3$ (AN = 23.1), 939 cm^{-1} in EtOH (AN = 37.1), and 930 cm^{-1} in MeOH (AN = 41.3). Plots of the S=O stretching vibrations of PPh$_4$−**2** vs the AN values of several solvents showed a linear relationship (Figure 2). This represents a quite different behavior from the case of complex **3**, which did not show any appreciable solvent effects (1070 cm^{-1} in acetone, 1069 cm^{-1} in MeCN, 1067 cm^{-1} in CHCl$_3$, 1069 cm^{-1} in 2-PrOH (AN = 33.5), and 1070 cm^{-1} in MeOH). Unexpectedly, the ν(S=O) for **2** (930 cm^{-1}) in MeOH is near the analogous value for native Fe-type NHase (910 cm^{-1}).[47] This observation may indicate that the electronic environment of complex **2** is similar to that of native

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NHase. Additionally, the sulfenyl oxygen may induce significant interactions with protic solvent molecules that have a higher acceptor number. The interaction of the S=O moiety with a proton was also investigated at low temperature by Kovacs et al.,[48] who described that addition of HBF_4 as a proton source into complex $[Fe^{III}(ADIT)(ADIT-O)]^+$ in MeCN produces a clear spectral change with isosbestic points. The conclusion was that the S=O group could function as a proton acceptor to form an S-coordinated S−OH group. Additional support for this proposal was obtained by observation of reversible spectral changes upon addition of triethylammonium base into the solution.

In discussions of the crystal structure of Fe−NHase it has been reported that the sulfenyl and sulfinyl groups interact with guanidinium groups of arginine moieties through hydrogen bonds between a sulfenyl O atom and a proton of arginine $N(\beta Arg56)$, $SO\cdots(H)N_{Arg} = 2.68$ Å, and between sulfinyl $O(\alpha Cys112-SO_2(H))$ atoms and protons of arginines $N(\beta Arg56)$ and $N(\beta Arg141)$, $SO\cdots(H)N_{Arg} = 2.86$ and $SO\cdots(H)N_{Arg} = 2.89$ Å.[10] Recently, Noguchi et al. described the existence of an interaction of the Fe−SO(H) moiety with a proton by monitoring corresponding frequency shifts using a native Fe−NHase and ^{34}S-labeled mutants. These results were discussed in comparison with the DFT calculations using simple model molecules.[47] The conclusion was that although Fe−SO(H) is in a deprotonated form (Cys-SO−) in biological systems, the possibility of the existence of the protonated form could not completely be excluded. It was further suggested that the deprotonated $\alpha Cys112-SO_2^-$ and $\alpha Cys114-SO^-$ participate in hydrogen bonds with the protons of $\beta Arg56$ and/or $\beta Arg141$ to form a reactive cavity at the interface of the α and β subunits and that at least one of these groups is hydrogen bonded to a reactant water molecule to increase its basicity in order to facilitate the nucleophilic attack on the iron-bound nitrile substrate.

The effect of the sulfenyl group on coordination of the *t*BuNC molecule in the solution state was studied by measuring the N≡C stretching frequencies, $\nu(N≡C)$, for **1**−**3** in MeCN solution. As expected from the results described above, the $\nu(N≡C)$ bands shift to a high-energy region according to the following trend: **1**(2169 cm^{-1}) < **2**(2190 cm^{-1}) < **3**(2213 cm^{-1}). Each of these values is larger than that of metal-free *t*BuNC molecule (2135 cm^{-1}). Considering that the HOMO of the *t*BuNC molecule is an antibonding σ^* orbital, the higher energy shift of $\nu(N≡C)$ observed upon oxidation of sulfur atoms may indicate stronger coordination of the *t*BuNC axial ligand. These facts suggest that the stability increases in the order of **1** < **2** < **3**.

Thus, we can conclude that the SO group is nucleophilic and that the greater stability of the axial coordination of *t*BuNC molecule is achieved by oxidation of sulfur. These observations suggest that the sulfenyl oxygen in NHase functions as an activator of water molecules and/or nitrile substrates through its nucleophilic interactions.
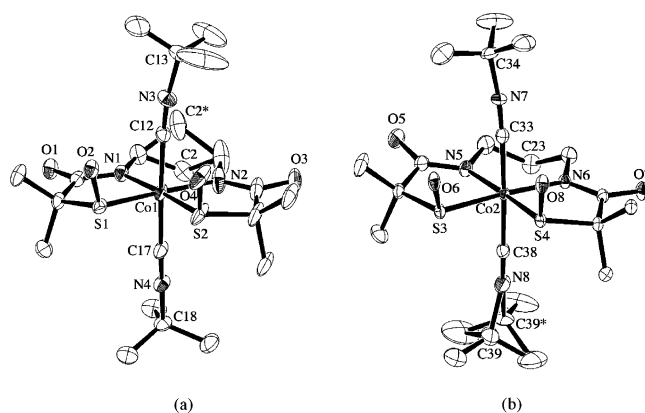
(48) Lugo-Mas, P.; Dey, A.; Xu, L. K.; Davin, S. D.; Benedict, J.; Kaminsky, W.; Hodgson, K. O.; Hedman, B.; Solomon, E. I.; Kovacs, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 11211−11221.

Figure 3. ORTEP drawings of two independent anion parts of crystal **2**, which are drawn at the 50% probability level. H atoms are omitted for clarity.
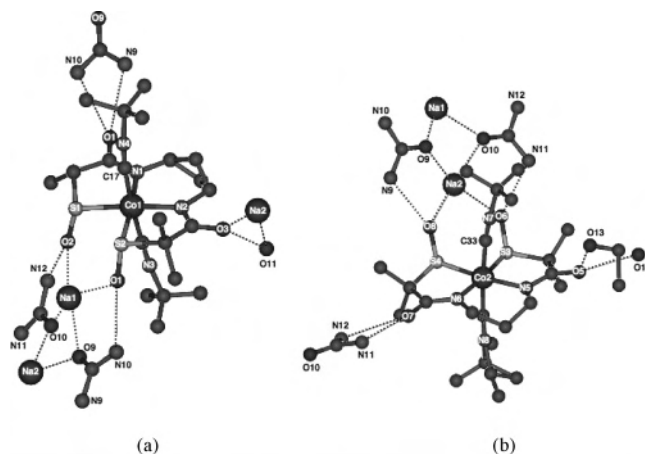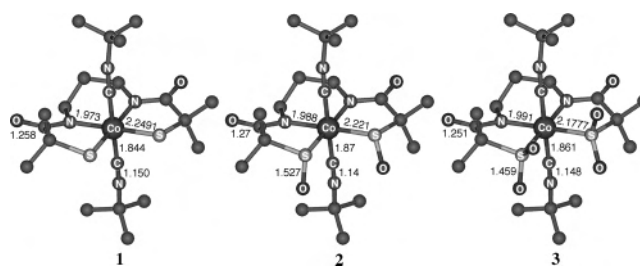

Figure 4. ORTEP drawings of two independent molecules for crystal **2** showing the hydrogen-bond and electrostatic interaction networks around the C=O and S=O groups.

Crystal Structure of $Na[Co^{III}(L_{C=O}:N_2(SO)_2)(tBuNC)_2]$ (2). Recrystallization of complex **2** in an ether/EtOH mixed solution afforded a single crystal suitable for X-ray structure analysis. There are two crystallographically independent molecules of **2** in the unit cell. The ORTEP views of two anion parts are shown in Figure 3, and the crystal structure showing hydrogen-bonding networks in the unit cell is given in Figure 4. Selected bond lengths and angles are listed in Table 2. One of the two independent complexes included a disordered axially coordinated *t*BuNC molecule (Figure 3 (right)). Another complex also has a disordered propyl chelate ring (Figure 2 (left)). The two independent molecules were found to be linked to each other via the carbonyl oxygens, sulfenyl oxygens, sodium cations, water molecules, ethanol, and urea molecules through hydrogen bonds and electrostatic interactions. The sulfenyl oxygens are linked to a Na^+ ion and an N−H group of a urea molecule for both species: $SO\cdots Na^+ = $ av. 2.35 Å and $SO\cdots(H)N = $ av. 2.92 Å. A similar network containing sulfenyl oxygen atoms was also found in the crystal structure of an N_2S_2-type Co(III) complex with two sulfenate groups, $Na[Co(L-N_2SOSO)-(tBuNC)_2]\cdot urea\cdot 2H_2O$, reported previously by Chottard et al.: $SO\cdots Na^+ = 2.32$ Å and $SO\cdots(H)N = $ av. 2.90 Å.[24] This is quite different than the case of $PPh_4[Co(L:N_2(SO_2)_2)-(tBuNC)_2]$ (**3**), where a hydrogen bond with the S−O oxygen

Table 2. Selected Bond Lengths [Å] and Angles [deg] for
Na[Co(L$_{C=O}$:N$_2$(SO)$_2$)(tBuNC)$_2$]·urea·2H$_2$O·EtOH (**2**)

Co1−N1	1.990(8)	O1−C4	1.28(1)
Co1−N2	1.99(1)	O3−C8	1.26(2)
Co2−N5	1.989(7)	O5−C25	1.27(1)
Co2−N6	1.982(7)	O7−C29	1.253(10)
Co1−S1	2.219(3)	S1−O2	1.514(7)
Co1−S2	2.226(3)	S2−O4	1.527(8)
Co2−S3	2.226(2)	S3−O6	1.540(7)
Co2−S4	2.213(2)	S4−O8	1.527(7)
Co1−C12	1.87(1)	O1···N9	2.94
Co1−C17	1.89(1)	O1···N10	2.92
Co2−C33	1.865(10)	O3···Na2	2.31
Co2−C38	1.866(9)	O3···O11	2.63
N1−C4	1.31(1)	O5···O13	2.69
N2−C8	1.30(2)	O5···O14	2.92
N5−C25	1.33(1)	O7···N11	2.91
N6−C29	1.30(1)	O7···N12	2.96
N1−Co1−N2	97.7(4)	S2−Co1−N2	87.4(3)
N5−Co2−N6	98.5(3)	S4−Co2−N6	86.8(2)
S1−Co1−S2	87.1(1)	C12−Co1−C17	176.1(5)
S3−Co2−S4	86.88(9)	C33−Co2−C38	175.7(4)
S1−Co1−N1	87.8(3)	Co1−C12−N3	173(1)
S3−Co2−N5	87.9(2)	Co2−C33−N7	174.9(8)
S1−Co1−N2	174.4(3)	Co1−C17−N4	173(1)
S3−Co2−N6	173.6(2)	Co2−C38−N8	174(1)
S2−Co1−N1	174.9(3)		
S4−Co2−N5	174.1(3)		

Figure 5. Comparison of average bond parameters around the Co atoms for complexes **1**−**3**.

atoms was not identified.[28] The interactions of the sulfenyl oxygen with Na$^+$ ions and urea molecules may contribute to the stabilization of complex **2** because the sulfenyl group of complex **2** is easily oxidized to a sulfinyl group in DMF, while it is not in more protic solvent like MeOH. The sulfenyl moiety in complex **2** might be able to exist only in the presence of an electrophilic environment, which probably prevents the sulfenate species from further oxidation.

The bond lengths of the ligands coordinated to the Co-(III) atom are Co−S$_{SO}$ = av. 2.22 Å (2.219(3), 2.226(3), 2.226(2), and 2.213(2) Å), Co−N$_{amide}$ = av. 1.98 Å (1.990-(8), 1.99(1), 1.989(7), and 1.982(7) Å), and Co−C$_{iso}$ = av. 1.87 Å (1.87(1), 1.89(1), 1.865(10), and 1.866(9) Å). These values are generally in agreement with those of the previously reported complex Na[Co(L-N_2SOSO)(tBuNC)$_2$]·urea·2H$_2$O (Co−S$_{SO}$ = 2.223(4), 2.223(4) Å, Co−N$_{amide}$ = 1.94(1) and 1.95(1) Å, and Co−C$_{iso}$ = 1.84(2) and 1.83(2) Å).[24] The N−Co−N angles (97.7° (4), 98.5° (3)) for **2** are larger than those of Na[Co(L-N_2SOSO)(tBuNC)$_2$]·urea·2H$_2$O (93.5°). In contrast, the S−Co−S angles of **2** (87.1° (1), 86.88° (9)) were slightly smaller than those of Na[Co(L-N_2SOSO)-(tBuNC)$_2$]·urea·2H$_2$O (90.4°). The difference is probably caused by a difference in the planarity of the six-membered chelate ring caused by differences in the arrangements of two amidate carbonyl groups between **2** and Na[Co(L-N_2SOSO)(tBuNC)$_2$]·urea·2H$_2$O. However, the bond parameters of the Co ligands for complex **2** were significantly different from those of complexes **1** and **3**, which have an increased extent of oxidation of sulfur atoms (Figure 5). The averaged Co−S bond lengths are shorter according to the following trend: **1** (2.25 Å) > **2** (2.22 Å) > **3** (2.18 Å). On the other hand, the Co−N$_{amide}$ bond length increases according to the following trend: **1** (1.973(2) Å) < **2** (av. 1.98 Å, 1.982(7)−1.990(8) Å) < **3** (av. 1.992 Å, 1.986(2)−1.996-

(2) Å). This lengthening is explained by the trans effect of S < SO < SO$_2$, where the SO$_2$ group has a larger trans effect.[49,50] On the other hand, the effect of oxidation of the sulfur atom did not induce a significant change in the Co−C(tBuNC) bond lengths at the axial sites: **1** (1.844(3) Å) < **2** (av. 1.87 Å (1.865(10), 1.866(9), 1.87(1), 1.89(1) Å)) ≈ **3** (av. 1.86 Å (1.874(3), 1.847(3) Å)).

The S−O bond for **2** is 1.527 Å, a length similar to that of Na[Co(L-N_2SOSO)(tBuNC)$_2$]·urea·2H$_2$O (1.50 Å), indicating that the S−O bond has double-bond character. The S−O bond for **2** is slightly longer than that of **3**, which agrees well with the values of S−O stretching frequencies described below (974 cm^{-1} for **2** and 1214 and 1071 cm^{-1} for **3**). The differences in N≡C bond lengths of the coordinated tBuNC molecules were almost not detected for **1** (av. 1.150 Å), **2** (av. 1.14 Å), and **3** (av. 1.148 Å), although there are some differences in the Co−C(tBuNC) bonds among these complexes. This is because the N≡C bond may not be discernible in the crystal structure.

Thermal Analyses for 1−3. TG measurements were carried out for **1**−**3** in the solid state to investigate the coordination behavior of the axial ligand. All complexes undergo three step degradations in the temperature range of 24−300 °C until the complexes are decomposed. The first step is due to a phase transition of the crystal, and the third step is a result of combustion. The second degradation step occurring for **1** at 151 °C, **2** at 170 °C, and **3** at 181 °C is due to release of two tBuCN molecules as judged from the weight loss of the complex. The temperature point at which this second degradation step occurs increases with the extent of oxidation of the sulfur atoms, indicating that of the stability trend of the complex is **1** < **2** < **3**. This trend supports the results obtained for complexes in solution. These behaviors are explained by the electron-withdrawing character of the oxygen atom; complexes **2** and **3** with the sulfenyl and sulfinyl groups having one and two oxygen atoms, respectively, increase the Lewis acidity of the Co(III) atom to stabilize the Co−C(tBuNC) bond relative to complex **1**, which lacks an oxygen atom. This is consistent with the fact that the axial ligands of **1** are easily released in organic solvents.

Infrared Spectra in the Solid State. Solid-state IR spectra of complex **2** were measured as KBr pellets. The spectra

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(50) Kastner, M. E.; Smith, D. A.; Kuzmission, A. G.; Cooper, J. N.; Tyree, T.; Yearick, M. *Inorg. Chim. Acta* **1989**, *158*, 185−199.

exhibited bands at 974, 1548, 1672, and 2192 cm^{-1} that were assignable to S=O, C=O ($L_{C=O}$:N$_2$(SO)$_2$), C=O(urea), and N≡C stretching vibrations, respectively. The signal corresponding to the C=O stretching vibration observed at 1548 cm^{-1} was split multiply. This is interpreted in terms of the existence of several interactions with Na$^+$, urea, and/or water molecules, as shown in the crystal structure (Figure 4). The features corresponding to the ν(S=O) and ν(N≡C) bands were observed in almost the same range as those (965 cm^{-1} for ν(S=O), 2195 (and 2375) cm^{-1} for ν(N≡C)) for the previously reported complex, Na[Co(L-*N₂SOSO*)-(*t*BuNC)$_2$]·urea·2H$_2$O, although there are some differences in ligand structures and hydrogen-bonding networks.[24]

It is very important to examine the influence of oxidation of sulfur atoms of the ligands, $L_{C=O}$:N$_2$S$_2$ and $L_{C=O}$:N$_2$(SO)$_2$, on the behavior of the axial and equatorial ligands. IR data of complexes **1** and **3** were previously reported as follows: the C=O and N≡C stretching bands were detected at 1534 and 2176 cm^{-1} for **1** and 1552 and 2199 cm^{-1} for **3**, respectively. The ν(C=O) and ν(N≡C) values for **2**, 1548 and 2192 cm^{-1}, respectively, are between those of **1** and **3**: **1** < **2** < **3** for ν(C=O) and **1** < **2** < **3** for ν(N≡C). The former behavior suggests that elongation of the C=O bond by oxidation of the sulfur atoms of the ligands occurs, although evidence of this C=O bond elongation was not detected in the C=O bond lengths in the crystal structures.

The ν(N≡C) band also showed a significantly higher wavenumber shift in the order of **1** < **2** < **3** with increasing oxidation of the sulfur atom, although this shift was not reflected in the N≡C bond lengths of the crystal structures. The values are all larger than those of the metal-free *t*BuNC molecule (2135 cm^{-1}). Oxidation of S to SO caused a shift in the N≡C frequency by 16 cm^{-1} to the higher energy region, indicating that the Co$-$C$_{iso}$ bond was strengthened in **2** relative to **1**. Further oxidation of SO to SO$_2$ also caused a 7 cm^{-1} higher energy shift in the N≡C frequency. Such a higher energy shift is also observed in the case of oxygenation of S (2170 cm^{-1}) to SO$_2$ (2210 cm^{-1}) using a four-coordinate Co(III) complex with a mixed carboxamido *N*-thiolato S donor set.[46] Considering that an antibonding σ^* orbital is a HOMO of the *t*BuNC molecule, these behaviors are easily understandable; the stronger σ-donation of the *t*BuNC molecule to the Co(III) atom with higher Lewis acidity promotes donation of electron density from the σ^*-orbital character of the N≡C bond to the Co atom to strengthen the Co$-$C bond. This finding implies that the increase in the extent of oxidation of sulfur atoms promotes stronger coordination of the *t*BuNC molecule. Since a nitrile molecule, which is a substrate of NHase, has a structure which is isoelectronic with isocyanide, the nitrile is also expected to be stabilized by oxidation of the sulfur atoms. The strong binding of a nitrile compound to the Co atom may induce polarization of the C≡N bond that is attacked by an activated water molecule.

In a biological NHase system the SO group has been reported to play an important role in its enzymatic reactivity because NHase is deactivated by oxidation of the SO group

to SO$_2$.[13] In the section describing solution-state IR spectra we argue that the SO group has sufficient nucleophilicity to activate a water molecule or a nitrile substrate. However, at this point we demonstrated that oxidation of the sulfur atom strengthens coordination of the *t*BuNC molecule. Complexes **2** and **3** with SO and SO$_2$ groups apparently have a stronger coordination of the axial *t*BuNC ligand than **1** which has a non-oxidized sulfur atom, which is advantageous from the viewpoint of activation of a water and/or nitrile molecule as discussed above. Oxidation of the sulfur atom raises the Lewis acidity of the Co atom. Recently, Himo et al. performed theoretical calculations on the first-shell mechanism for the hydration of nitrile compounds by Fe-type NHase and proposed that the role of the metal ion is to provide electrostatic stabilization of the anionic imidate intermediate rather than to function as a Lewis acid.[51] The calculations have indicated that the sulfenyl oxygen of Cys114-SO$^-$ might function as a catalytic base to abstract a proton from the attacking water molecule. Our current results are supported by this theoretical study.

Theoretical Calculations. The spectroscopic, thermogravimetric, and structural characterizations of complex **2** described above were compared with those of complexes **1** and **3**. To understand the stability of complex **2** and the role of the sulfenyl group, DFT calculations were performed for **1**−**3** on the basis of their crystal structures. Previously, Richard et al. reported the spin-dependent properties of open-shell Fe(III) complexes with non-oxygenated sulfur atoms on the nitrile hydratase function by DFT calculation.[52] However, they did not treat the Co(III) complexes and did not discuss the effect of oxygenation on the electronic structures of the complexes. Here, we calculated three Co-(III) complexes with ($L_{C=O}$:N$_2$S$_2$), ($L_{C=O}$:N$_2$(SO)$_2$), and ($L_{C=O}$:N$_2$(SO$_2$)$_2$), (PPh$_4$[Co^{III}($L_{C=O}$:N$_2$S$_2$)(*t*BuNC)$_2$] ([Na[Co^{III}-($L_{C=O}$:N$_2$(SO))(*t*BuNC)$_2$], and PPh$_4$[Co^{III}($L_{C=O}$:N$_2$(SO$_2$)$_2$)(*t*BuNC)$_2$]]) and discussed the relationship between the nitrile hydratase function and the oxygenation effect.

Electron densities of complexes **1**−**3** were estimated by Milliken population analyses. The net charges on the selected atoms are listed in Table 3. The extent of oxidation of sulfur atoms of the ligands was detected as a remarkable change in the charges of the Co atoms, although a larger variation was detected on S atoms. The charges on the Co(III) centers were estimated to be +1.466, +1.536, and +1.542 for **1**, **2**, and **3**, respectively, indicating that the Lewis acidity of the Co(III) center increases with increasing extent of oxidation of sulfur atoms. Similar behavior has been found also by Darensbourg et al. using Ni(II) complexes with two amine N and two thiolate S atoms. They showed that the Lewis acidity on the metal atom was increased with the oxygenation of S atoms through a loss of σ- and π-donor abilities of the sulfur atoms,[53] but they did not discuss their stabilities. The

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(53) Grapperhaus, C. A.; Darensbourg, M. Y. *Acc. Chem. Res.* **1998**, *31*, 451−459.

Table 3. Electric Charges on Atoms Estimated by Mulliken Population Analysis for PPh$_4$[Co(L$_{C=O}$:N$_2$S$_2$)(tBuNC)$_2$] (**1**), Na[Co(L$_{C=O}$:N$_2$(SO)$_2$)(tBuNC)$_2$] (**2**), and PPh$_4$[Co(L$_{C=O}$:N$_2$(SO$_2$)$_2$)(tBuNC)$_2$] (**3**)

		1	2	3
	Co	1.466	1.536	1.542
S–O	S1	-0.305	0.269	0.983
	S2	-0.305	0.308	0.987
	S$_{average}$	-0.305	0.288	0.985
	O1	–	-0.677	-0.721
	O2	–	-0.696	-0.718
	O3	–	–	-0.723
	O4	–	–	-0.718
	O$_{average}$	–	-0.686	-0.720
tBuNC	C1	0.084	0.096	0.075
	C2	0.083	0.071	0.069
	C$_{average}$	0.084	0.084	0.072
	N3	-0.354	-0.333	-0.318
	N4	-0.355	-0.335	-0.312
	N$_{average}$	-0.355	-0.334	-0.315
Amidato N	N1	-0.490	-0.490	-0.497
	N2	-0.490	-0.497	-0.492
	N$_{average}$	-0.490	-0.494	-0.495
C–O	O5	-0.507	-0.489	-0.482
	O6	-0.509	-0.503	-0.489
	O$_{average}$	-0.508	-0.496	-0.486
	C3	0.325	0.300	0.317
	C5	0.324	0.329	0.317
	C$_{average}$	0.325	0.315	0.317
(CH$_3$)$_2$$\underline{C}$	C4	-0.073	-0.083	-0.109
	C6	-0.073	-0.078	-0.103
	C$_{average}$	-0.073	-0.081	-0.106
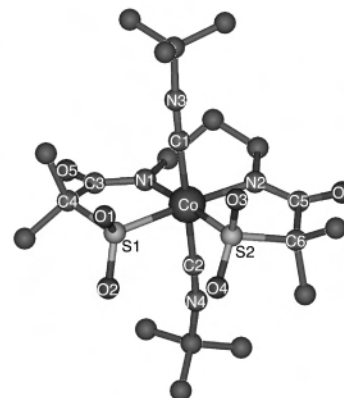
increase in the Lewis acidity is consistent with the measurements of thermal stability described above.

The C and O atoms of the carbonyl groups and the C atom linked to the sulfur atoms of the ligands (−N−C(=O)−C−S−) show alternating charge density distributions. The coordinated nitrogen atoms were not significantly affected by the extent of oxidation, although the Co−N bonds were lengthened by the trans effect in their crystal structures: N(−0.490)−C(+0.325)(=O(−0.508))−C(−0.073)−S(−0.305) for **1**, N(−0.494)−C(+0.330)(=O(−0.496))−C(−0.081)−S(+0.289) for **2**, and N(−0.495)−C(+0.317)(=O(−0.486))−C(−0.106)−S(+0.985) for **3**. The electronic effect of oxidation appeared on the C atoms adjacent to S atoms rather than the N atoms. This result may imply that the effect of extent of oxidation is imparted through the sulfur atom. The differences of charges of C and O atoms may concern the bonding nature of C−O groups because the order of C=O stretching frequencies, **1** (1534 cm^{-1}) < **2** (1548 cm^{-1}) < **3** (1552 cm^{-1}), are well correlated with the order of differences of their charges, 0.833 (**1**) > 0.826 (**2**) > 0.803 (**3**). This is

explained as follows: a larger difference in the value implies larger polarization of the bonded atoms, resulting in weakening of the C−O bond.

The charges on the C and N atoms of the axially coordinated *t*BuNC molecule are also alternating changed: (Co-(+1.466)−)C(+0.084)≡N(−0.354) for **1**, (Co(+1.536)−)-C(+0.084)≡N(−0.334) for **2**, and (Co(+1.542)−)C-(+0.072)≡N(−0.315) for **3**. Interestingly, the change in the charge density of the C atom was quite small in spite of the large change in the charge density of the Co atom. The charge on the N atom showed a significant reduction in negative charge. This can be explained by reduction of π-back-donation from Co to the C atom of the *t*BuNC molecule that was induced by the electron-withdrawing oxygen atom bound to sulfur. This speculation was also elucidated from estimation of π-back-donation to the Co−C and Co−S bonds using the DFT method as follows. The extent of electronic π-back-donations from 3d$_{zx}$ and 3d$_{yz}$ on the Co(III) ion to two π*-orbitals of the C atom, which were estimated by second-order perturbation energy $E(2)$ for the three complexes, demonstrated the following trend: **1** (35.1 kcal/mol) > **2** (30.52 kcal/mol) > **3** (29.07 kcal/mol). The extent of electronic π-back-donations from 3d$_{yz}$ and 3d$_{zx}$ orbitals on the Co(III) to virtual orbitals on S increased according to the following trend: **1** (1.64 kcal/mol) < **2** (3.14 kcal/mol) < **3** (5.48 kcal/mol).

These calculation results may give an understanding of the stabilities and ν(N≡C) stretching vibration values of complexes **1**−**3**. Oxidation of the sulfur atoms stabilize the Co-*t*BuNC bonds through the π-electron delocalization of the N≡C−Co−S=O bond in the order of **1** < **2** < **3**. This is, the π-back-donation from the Co dπ-orbital to the S π*-orbital was enhanced by oxygenation (S → SO → SO$_2$), which lowered the π-back-donation from the Co dπ-orbital to the π*(N≡C)-orbital. This lowering will induce the increase in the N≡C bond strength, which appeared as an increase in the ν(N≡C) stretching vibration values of complexes **1**−**3** (**1** (2176 cm^{-1}) < **2** (2192 cm^{-1}) < **3** (2199 cm^{-1})). The increase in the π-back-donation from the Co dπ-orbital to the S π*-orbital by oxygenation (S → SO → SO$_2$) also increased the positive charge density on the metal atom, which enhanced σ-donation from the σ*(N≡C)-orbital to the metal ion, which was also reflected as the strength of the C≡N bond.

Summary

We synthesized a new Co(III) complex **2** with sulfenate sulfur and amidate nitrogen atoms in the equatorial plane and with two *t*BuNC molecules at the axial sites in order to understand the role of the sulfenate group and the effect of oxidation of the coordinated sulfur atoms. Spectroscopic and structural features were discussed through comparison with those of complexes **1** and **3** reported previously. As a result, the following insights have been obtained. (i) Oxidation of sulfur atoms of the ligands increases the strong coordination of *t*BuNC molecules to the Co(III) atom. This higher stability has been explained by an increase in the Lewis acidity of the Co(III) ion and by electronic delocalization through the N≡C−Co−S=O bonds induced by the electron-withdrawing oxygen. (ii) The sulfenyl group is quite sensitive to the protic solvent, although the sulfinyl group is unresponsive for all solvents used. These facts suggest that the sulfenyl oxygen has nucleophilic character and contributes to activation of a water molecule and/or a nitrile substrate in the NHase mechanism. The arginine residues observed in the crystal structures of NHase are likely to play a role in regulation of the active site through binding interactions with the sulfenyl oxygen.

Here we propose the following biological implication for NHase function. Oxygenation of the sulfur atom of the ligand to SO or SO$_2$ increased the Lewis acidity of the central Co-(III) ion, which derived the following two possibilities. One is the case of coordination of a water molecule to the metal ion. The pK_a of the coordinated water molecule will be lowered by the oxygenation, and then the generated metal-bound hydroxide will attack the nitrile compound to promote catalytic hydration. Another is the case of coordination of the nitrile compound. The SO group with a nucleophilic property activates the water molecule by H-atom abstraction, and then the activated water molecule attacks the coordinated nitrile compound to give the amide compound.

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