A Bis(carboxamido-*N*)diisocyanidobis(sulfenato-*S*)cobalt(III) Complex, Model for the Post-Translational Oxygenation of Nitrile Hydratase Thiolato Ligands

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A square-planar cobalt(III) complex with an N_2S_2 bis(carboxamido-*N*)dithiolato tetradentate ligand was oxygenated by H_2O_2·urea, after axial coordination of *tert*-butyl isocyanide, to the first bis(carboxamido-*N*)bis(sulfenato-*S*)cobalt(III) complex.

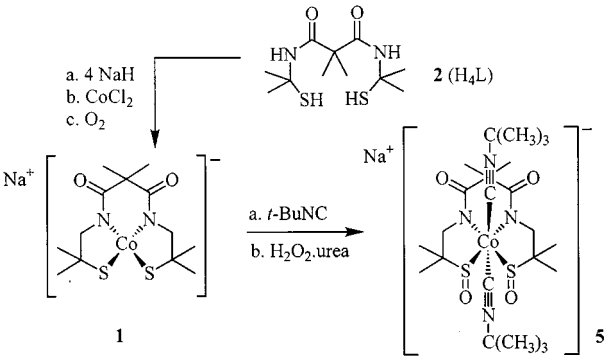
Introduction

Nitrile hydratases (NHases) are non-heme iron or non-corrinoid cobalt-containing enzymes which catalyze the hydration of nitriles to amides.[1a] Recent X-ray studies of NHases indicated that the iron center is bound to two peptide amide nitrogen atoms and three sulfur atoms from cystein-thiolate, cystein-sulfinic and cystein-sulfenic moieties, the latter two resulting from post-translational oxidation.[1a,1b] The two nitrogen atoms and the two sulfur atoms of the modified cystein groups belong to a planar tetradentate eleven-atom ligand from a Cys-Ser-Cys sequence. EXAFS data suggest that the iron and cobalt NHases have similar metal sites.[1c] However, there is no evidence whether post-translational oxidation of cystein-thiolates also occurs in cobalt sites.

A few Co^{III} complexes with both thiolato and carboxamido-*N* ligands have recently been reported as synthetic models of NHases.[2] Two of them have been oxidized by H_2O_2 to give the sulfinato-*S* species,[2b] but no sulfenato-*S* derivative was characterized.

We have recently reported[3] the first example of dioxygen oxidation of a (carboxamido-*N*)(thiolato)Fe^{III} complex $[FeN_2S_3]^{2-}$ into a sulfinato-*O* species $[FeN_2S_2OSO]^{2-}$. The sulfinato-*O* coordination suggested a preferred oxidation of the axial thiolato ligand, compared to that of the thiolates in the equatorial N_2S_2 plane. To address this question in the case of Co NHases, we have synthesized complex **1** ($[Co^{III}L]^-$) from the deprotonated ligand **2** (H_4L)[4] (Scheme 1). The structure of **1** was determined by X-ray diffraction.[5]

The square-planar complex **1** is stable in air, in the solid state and in solution. Its reaction with excess H_2O_2 or H_2O_2·urea leads to decomposition. We have shown by UV/Vis spectroscopy and EXAFS studies, that two CN^- or *t*BuNC ligands are able to bind at the axial positions of **1**, leading to the hexacoordinated diamagnetic complexes $[CoL(CN)_2]^{3-}$ (**3**) and $[CoL(tBuNC)_2]^-$ (**4**), respectively[5]. Here we report the oxygenation of the thiolato ligands in **4**, giving the corresponding bis(sulfenato-*S*) complex **5**.



Scheme 1. Synthesis of complexes **1** and **5**

Results and Discussion

At variance with **1**, complexes **3** and **4** undergo fast oxygenation reactions in aerated solution, giving a mixture of presumably sulfinato (-*O* or -*S*) and sulfenato (-*O* or -*S*) species. The addition of H_2O_2·urea to a solution of **1** in the presence of excess *t*BuNC gave the orange complex **5** {$Na[Co^{III}(L\text{-}N_2SOSO)(tBuNC)_2]$·urea·$2H_2O$} which precipitated spontaneously (Scheme 1). The X-ray structure of **5** (Figure 1) reveals the oxygenation of the two thiolato to sulfenato-*S* ligands. It is noteworthy that the urea molecules contribute to the crystal lattice through binding to oxygen atoms of the sulfenato-*S* and carboxamido-*N* ligands and by interaction with the Na^+ ions (Figure 2). Complex **5** contains two axial isocyanide ligands in a distorted octahedron. This suggests that the oxygenation occurred thanks to the formation of **4** {$Na[CoL(tBuNC)_2]$}. The presence of one Na^+ per Co in the unit cell of **5** supports a cobalt(III) state. The Co−N bond lengths are similar to those reported for carboxamido-*N* ligands in hexacoordinated Co^{III} complexes,[2] but are 0.07 Å longer than those in the square-

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planar complex **1**. Very few complexes have been reported with structurally characterized sulfenato-*S*−cobalt(III) bonds; their lengths are in the range 2.198−2.253 Å,[6] in agreement with the 2.223 Å in **5**. The two oxygen atoms of the sulfenato-*S* ligands are on the same side of the N_2S_2 plane, leading to a slight asymmetry of **5**. To our knowledge, only two cyclopentadienyl compounds with isocyanido-*C*−cobalt(III) bonds are known with C−Co^{III} bond lengths of 1.83 and 1.79 Å,[7] comparable to the 1.83 Å in **5**. The isocyanides are linearly coordinated to the metal center, with the following angle values Co(1)−C(14)−N(4) = 175.1°, Co(1)−C(19)−N(3) = 179.5°, C(14)−N(4)−C(15) = 176.5°, C(19)−N(3)−C(20) = 176.6° and a 1.14 Å C−N bond length. This agrees with a C−N triple bond description. The IR spectrum (KBr pellet) of **5** shows the characteristic coordinated carboxamido-*N* ν_{CO} frequency at $\tilde{\nu}$ = 1555 cm^{-1} and a strong absorption at $\tilde{\nu}$ = 965 cm^{-1} corresponding to the ν_{SO} stretching of coordinated sulfenato-*S* ligands.[6b] The weak symmetric and strong asymmetric ν_{CN} stretching frequencies of the isocyanide groups are increased from $\tilde{\nu}$ = 2140 cm^{-1} to 2375 and 2195 cm^{-1} upon coordination, indicating a higher bond order in **5** than in the free ligand, fitting the C−N triple bond description and the σ-donor character of the isocyanide groups which dominates their potential π-acceptor contribution.

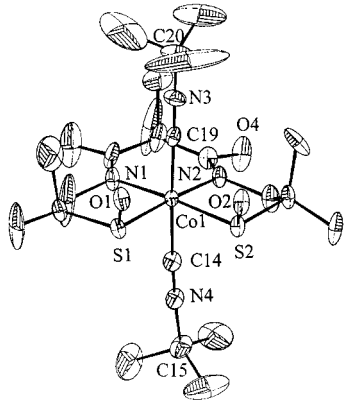

Figure 1. CAMERON representation (ellipsoid at 20% probability level) of **5**; H atoms are omitted for clarity; selected bond lengths [Å] and angles [°]: Co(1)−S(1) 2.223(4), Co(1)−S(2) 2.223(4), Co(1)−N(1) 1.94(1), Co(1)−N(2) 1.95(1), Co(1)−C(14) 1.84(2), Co(1)−C(19) 1.83(2), C(14)−N(4) 1.14(2), C(19)−N(3) 1.14(2); S(1)−Co(1)−S(2) 90.4(1), S(1)−Co(1)−N(1) 87.9(4), N(1)−Co(1)−N(2) 93.5(5), C(14)−Co(1)−C(19) 177.5(6), C(14)−N(4)−C(15) 176.5(17), C(19)−N(3)−C(20) 176.6(20)

The orange solutions of **5** in polar solvents (alcohols, DMSO, DMF, H_2O) are stable in air and the complex appears hexacoordinated with diamagnetic low-spin Co^{III} (Evans method). The electrospray mass spectrum exhibits peaks at *m*/*z* = 559 ([M − Na^+]$^-$) and at *m*/*z* = 476 ([M − Na^+ − *t*BuNC]$^-$), and weaker ones at *m*/*z* = 575 and 492, suggesting partial oxidation of these two species during the mass spectrometry analysis.

Whereas the oxidation of (thiolato)Co^{III} complexes by H_2O_2 is well known,[8] there are only two examples of oxygenation of bis(carboxamido-*N*)(dithiolato)Co^{III} complexes which give the bis(sulfinato-*S*) species.[2b] Sulfenato-*S* complexes are usually difficult to isolate due to their further oxidation to the sulfinato-*S* derivatives. The isolation of **5** was likely due to its precipitation, preventing further oxidation. The oxidation of the thiolato ligands is believed to start by nucleophilic attack on dioxygen or H_2O_2, as in the case of (thiolato)nickel complexes.[8b] The increase of the Co−S bond lengths upon addition of CN^- or *t*BuNC to **1**, to give **3** and **4** (2.13, 2.23 and 2.28 Å for **1**, **3** and **4**, respectively, according to our EXAFS studies)[5] shows that the thiolato ligands become much less tightly bound in the latter two and consequently more nucleophilic.

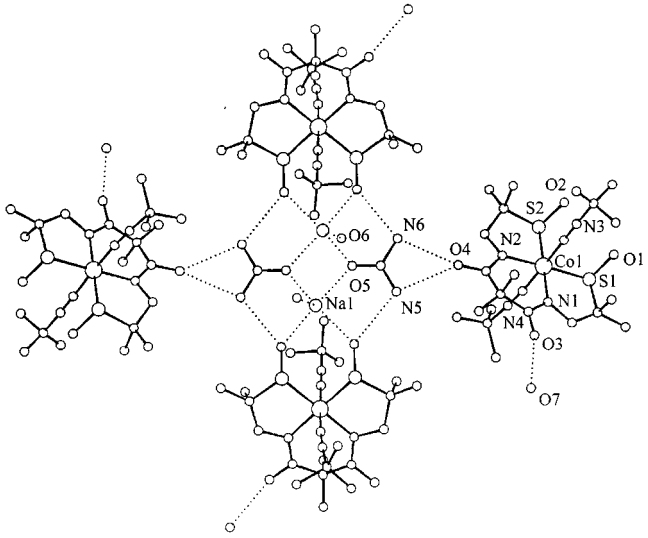

Figure 2. View of the Na^+/O interactions and hydrogen bonds stabilizing the crystal lattice of **5**·urea·$2H_2O$

A preliminary study of the catalytic hydration activity of **5** was conducted without acid or base in a 9:1 acetonitrile/water mixture at room temperature. After 24 h, acetamide was formed (2 turnovers) and **5** remained unchanged (^{1}H NMR criteria). No conversion was observed in the absence of **5**. Although **5** is not an efficient catalyst under these conditions, the hydration mechanism deserves interest. The known[2a] complex $[Co(PyPS)(OH)]^{2-}$ (with one pyridine, two carboxamido-*N* and one thiolato ligands in a plane, and the second thiolato ligand occupying an axial position) converts acetonitrile into acetamide only at 50°C and pH = 9.5 (Tris buffer), most probably via the Co^{III}-bound hydroxide ligand. It is noteworthy that **5** has no free coordination site that would allow either water or acetonitrile binding to Co^{III}. This suggests that the nucleophilicity of the coordinated sulfenato-*S* ligands[9] in **5** might play a crucial role in the hydration reaction of acetonitrile. Further work is in progress to test this hypothesis and its relevance to the case of NHase.

Conclusion

We have shown that H_2O_2·urea allowed the clean oxidation of the dithiolato complex **1** to its bis(sulfenato-*S*)Co^{III} derivative **5** after axial coordination of *tert*-butyl isocyanide.

It is the first example of a cobalt complex with a mixed carboxamido-*N* and sulfenato-*S* coordination sphere. These results suggest that the in-plane thiolates of the metallic site of Co NHases could undergo posttranslational oxidation, as observed for the Fe NHases. Such a reaction would require the presence of σ-donor ligands, a role which could be played by the axial cystein-thiolate and the external hydroxide bound to the metal center at the active site of the enzyme.[1] The coordinated sulfenate-*S* likely plays a key role in the activity of NHases.

Experimental Section

Complex 5: To a solution of **1** (42 mg, 0.1 mmol) and *t*BuNC (36 mg, 0.44 mmol) in degassed DMF (1 mL) cooled to 0°C was added a filtered solution of H_2O_2·urea (200 mg, 2.2 mmol) in degassed acetone (2 mL), leading to the formation of an orange precipitate. After 30 min at 0°C, the precipitate was isolated, dried under vacuum and dissolved in ethanol. Diethyl ether diffusion into this solution afforded **5** {Na[Co^{III}(L-N_2SOSO)(*t*BuN*C*)$_2$]·urea·$2H_2O$} as orange crystals (45 mg, 60%). − $C_{24}H_{48}CoN_6NaS_2O_7$ (678.73): calcd. C 42.47, H 7.13, N 12.38, S 9.45; found C 42.58, H 6.86, N 12.38, S 9.48. − UV/Vis (MeOH): λ_{max} (ε) = 460 (sh), 377 (9050), 297 (9380), 217 nm (12800 M^{-1} cm^{-1}). − IR (KBr) $\tilde{\nu}$ = 2375 (w, ν_{CN}) and 2195 (s, ν_{CN}), 1555 (s, ν_{CO}), 965 cm^{-1} (s, ν_{SO}). − ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 7.65 (s, 4 H, H_2O), 5.44 (s, 4 H, urea), 3.60 (d, 2 H, NCH_AH_B, J = 13.3 Hz), 3.09 (d, 2 H, NCH_AH_B), 1.39 (s, 18 H, isocyanides), 1.28, 1.17 and 1.12 (3 s, 3 × 6 H, methyl groups). − Electrospray MS (MeOH); *m*/*z* (%): 476 (100), 492 (22), 559 (86), 575 (16).

Crystal Data for 5·Urea·2H$_2$O: $C_{24}H_{48}CoN_6NaS_2O_7$, M = 678.73, orange hexagonal block 0.20 × 0.40 × 0.80 mm, monoclinic, space group $P2_{1/m}$, a = 10.488(3), b = 18.789(18), c = 21.474(13) Å, Z = 4, β = 98.14(6)°, V = 4189(5) Å^3, D_c = 1.076 g·cm^{-3}, T = 295 K, Mo-K_α radiation (λ = 0.71069 Å), 8208 reflections were measured, 2227 of which were used in all calculations (R_{int} = 0.04). The structure was solved by SHELXS-86 and refined by least-squares analysis using anisotropic thermal parameters, H atoms were introduced in calculated positions, with $F_o > 3\sigma(F_o)$, the programs CRYSTALS and CAMERON were used. R = 0.0935, R_W = 0.1071. It is noteworthy that the refinement factors R and R_W could not be reduced despite several attempts including measurements at lower temperatures. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-146921. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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