

Oxygenation of thiolates to *S*-bonded sulfinate in an iron(III) complex related to nitrile hydratase

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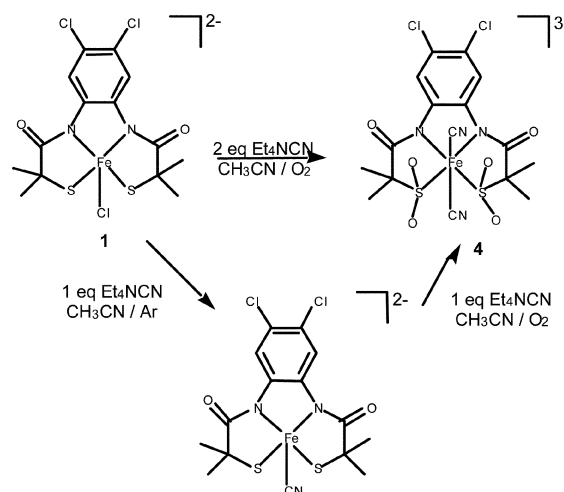
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Air oxidation of the iron(III) complex derived from (2-mercaptoisobutyl)-4,5-dichloro-*o*-phenylenediamine in the presence of Et₄NCN afforded the corresponding disulfinate species. With two carboxamido nitrogens *trans* to two *S*-bonded sulfinate, this complex mimics the disulfinate inactive form of Fe-NHase.

Nitrile hydratases (NHases) are bacterial enzymes that are essential for the industrial production of acrylamide.¹ They catalyze the hydration of nitriles to amides and contain either a low-spin non-heme iron(III) or non-corrinoid cobalt(III) center. The X-ray structures revealed that the endogenous ligands to the iron² or to the cobalt³ are two peptide amide nitrogens and three sulfurs arising from a highly conserved peptide sequence among NHases, CXYCSC. Two of the thiolates *trans* to two carboxamido nitrogens in the basal plane are post-translationally oxidized to sulfinate and sulfenate, while the third non-oxidized thiolate occupies an axial position *trans* to a water molecule or to a hydroxide anion. In contrast to Co-NHases, Fe-NHases have a high affinity for NO which is further released upon exposure to light.⁴ The role of NO and the origin of the post-translational modification of the protein remain unknown, even though the sulfenate seems likely to play a key role in the nitrile hydration process.⁵ Biomimic approach aims at reproducing Fe- and Co-NHases active site as well as their chemical reactivities. Whereas several Co-mimics containing sulfenate(s) and/or sulfinate(s) have been prepared,^{6a} only a few Fe-mimics are available.^{6a-c} Most of them contain non-oxidized thiolates and amine or imine nitrogens and very few introduce carboxamido nitrogens. In this latter case the thiolates can be oxidized to sulfinate(s) affording five-coordinate species with *O*-bonded sulfinate^{7a,b} and six-coordinate species with *S*-bonded sulfinate(s).^{7a,c} In no case are two oxidized thiolates *trans* to two carboxamido nitrogens as in Fe-NHase. Recently, we prepared a five-coordinate (Et₄N)₂[Fe(N₂S₂Cl)] complex with a square pyramidal structure, derived from (2-mercaptoisobutyl)-*o*-phenylenediamine N₂S₂H₄, which contains in the mean plane two thiolates *trans* to two deprotonated amides.⁸ This complex has a high affinity for NO and readily binds one NO molecule per iron leading to an air stable nitrosyl complex (Et₄N)[Fe(N₂S₂)(NO)] with a square pyramidal structure.⁸ We report here the synthesis and structural characterization of the six-coordinate (Et₄N)₃[Fe(N₂Cl₂(SO₂)₂)(CN)] disulfinate complex derived from an analogous ligand (2-mercaptoisobutyl)-4,5-dichloro-*o*-phenylenediamine.

The disubstituted ligand N₂S₂Cl₂H₄ was prepared in three steps, as described for (2-mercaptoisobutyl)-*o*-phenylenediamine.⁸ Two chloro substituents were introduced on the previous ligand to facilitate the crystallization of the final disulfinate complex. The ligand readily binds iron(III) in DMF at -15 °C in the presence of base as previously described for N₂S₂H₄.⁸

Complex **1** was characterized by elemental analysis.⁹ It exhibits similar spectroscopic characteristics to the structurally characterized (Et₄N)₂[Fe(N₂S₂Cl)] complex: i) UV-vis absorptions at 365 and 504 nm in CH₃CN, ii) a carbonyl stretching frequency ν_{CO} at 1590 cm⁻¹ as expected for coordinated carboxamido nitrogens, iii) a broad EPR spectrum in frozen CH₃CN at 20 K with *g* values at



4.58 and 1.96 typical of iron(III) with an *S* = 3/2 ground state. This complex is air stable. The axial chloro ligand can be readily exchanged with cyanide in acetonitrile with 1 equiv. of Et₄NCN. The cyano complex **2** (Et₄N)₂[Fe(N₂S₂Cl₂)(CN)] was first purified by LH20 chromatography eluted with methanol, then isolated as a red powder upon precipitation under argon from an acetonitrile solution by addition of diethyl ether. This complex is very hygroscopic and air sensitive, and has to be handled under argon. Its elemental analysis,¹⁰ as well as its IR spectrum displaying in addition to the ν_{CO} vibration at 1591 cm⁻¹ a stretching frequency at 2102 cm⁻¹, are in agreement with a monocyno species. Its UV-vis spectrum in CH₃CN exhibits two absorptions at 360 and 480 nm. Its EPR spectrum in CH₃CN glass shows signals at *g* = 4.38, 2.15 and 1.97 suggesting that at 20 K **2** is converted into two species characterized by two different spin states (*S* = 3/2 and *S* = 1/2). In agreement with this hypothesis, the spin concentration determined by double integration ratio of the signals at *g* = 4.38 (*S* = 3/2) and of those at 2.15 and 1.97 (*S* = 1/2) was equal to one. The six-coordinate species is assumed to be the dicyano complex **3** (Et₄N)₃[Fe(N₂S₂Cl₂)(CN)₂] and the *S* = 3/2 species the five-coordinate complex with a solvent molecule in the fifth position instead of the initial cyanide. All attempts to characterize the six-coordinate complex by low temperature UV-vis experiments have failed so far. Such a six-coordinate complex should exhibit a high wavelength absorption in the range 600 to 700 nm assigned to a thiolate-to-Fe(III) charge transfer band.^{7a} Decreasing the temperature of a solution of **2** in acetonitrile or in a mixture of CH₂Cl₂/CH₃CN down to -45 °C does not result in any change in the electronic spectrum, even in the presence of a large excess of cyanide. However, when **2** in acetonitrile was exposed to dioxygen overnight in the presence of 1 equiv. of Et₄NCN, the red solution turned green. The electronic spectrum of this solution exhibits a band at 704 nm, suggesting the formation of a six-coordinate species. Precipitation of the product afforded a green solid which shows in its IR spectrum a ν_{CN} vibration at 2104 cm⁻¹ and two new strong bands at 1166 and 1050 cm⁻¹. The two latter bands indicate

oxidation of the thiolato sulfurs into sulfates and the formation of an *S*-bonded sulfinate. The elemental analysis is in agreement with the formulation $(Et_4N)_3[Fe(N_2Cl_2\{SO_2\}_2)(CN)_2]$ for complex **4**.¹¹ Its EPR spectrum in frozen CH_3CN displays two broad bands at $g = 2.14$ and 1.96 indicating that the iron(III) is in a low-spin state. Crystals suitable for X-ray analysis† were obtained by vapor diffusion of diethyl ether in an acetonitrile solution of **4**. The crystal structure of the anion of **4** shown in Fig. 1 reveals an octahedral environment for the iron(III) with two *S*-bonded sulfinate *trans* to two deprotonated amide nitrogens as in NHase and two axially bound cyanides. Oxygenation of the thiolates does not result in a significant change of the nitrogen-to-iron bond lengths but a 0.05 \AA increase of the Fe–S distances is noted, with average values of 2.201 and 2.252 \AA in $[Fe(N_2S_2)(Cl)]^{2-}$ ⁸ and **4** respectively. Interestingly, the Fe–S(O)₂ distances compare well with that reported for NHase (2.25 \AA).²

We have previously described the oxidation of thiolates to sulfates in a related Co(III) complex.¹² Different oxidation products were characterized when *t*BuNC^{12a} or cyanide^{12b} were used as axial ligands, and dioxirane^{12a} or dioxygen^{12b} as oxidants. Obtention of a clean oxidation product from **1** requires several conditions: i) introduction of CN^- as axial ligand to promote oxygenation (**1** is stable under O_2), ii) presence of a second equivalent of cyanide in the reaction mixture, since **2** decomposes under a dioxygen atmosphere, iii) use of dioxygen as oxidant. Oxidations carried out with either dioxirane or H_2O_2 result in the destruction of the complex, probably caused by further oxidation of the ligand. While it is clear that in the cobalt series thiolate oxygenation occurs through a six-coordinate Co(III) complex,¹² we have no evidence for the involvement of such an iron(III) species in the oxygenation of **1** and **2**. The six-coordinate dicyano complex **3** was only observed by EPR at a very low temperature. Consequently it might exist in a very small amount at room temperature and be involved in the oxidation process yielding the *S*-bonded species as previously discussed by Mascharak *et al.*^{7a} However, an alternative hypothesis could be the isomerization of an unstable *O*-bonded sulfinate to a more stable *S*-bonded species. The relative orientation of the thiolates to the amides at the metal center may thus have a critical role on the oxidation mechanism, as shown herein.

To our knowledge, this work is the first fully characterized example of thiolate to *S*-bonded sulfinate oxidation mediated by dioxygen in a NHase model complex. The previously reported

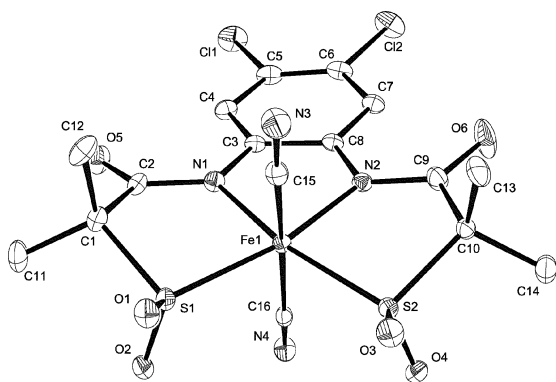


Fig. 1 Thermal ellipsoid plot (50% probability level) of the anion of **4**. H are omitted for clarity. Selected bond distances (in \AA) Fe1–N1, 1.960(2); Fe1–N2, 1.958(2); Fe1–S1, 2.2550(8); Fe1–S2, 2.2493(8); S1–O1, 1.467(2); S1–O2, 1.474(2); S2–O3, 1.473(2); S2–O4, 1.471(2); Fe1–C15, 1.965(3); Fe1–C16, 1.963(3); C15–N3, 1.148(4); C16–N4, 1.153(4). Selected bond angles (in deg) N1–Fe1–C16, 87.68(11); N1–Fe1–C15, 96.90(12); C15–Fe1–S1, 86.22(9); C15–Fe1–S2, 87.60(9); N2–Fe1–S1, 167.44(7); N1–Fe1–S1, 83.77(8); N1–Fe1–S2, 166.11(8); N2–Fe1–S2, 82.85(7); C16–Fe1–C15, 173.33(12); S2–Fe1–S1, 109.70(3).

dioxygen oxidation of thiolates afforded stable *O*-bonded species.^{7a,c} Finally, this complex can be considered as the first mimic of an inactive form of iron-NHase containing in the mean-plane two sulfinate instead of one sulfinate and one sulfenate, as recently described by Tsujimura *et al.*¹³

Notes and references

† Crystal data for **4**: $C_{42}H_{77}N_8O_6S_2Cl_2Fe$, $M_w = 981.009$, monoclinic, green crystal ($0.6 \times 0.3 \times 0.1 \text{ mm}^3$), $a = 20.0780(7)$, $b = 11.0660(2)$, $c = 22.5990(8) \text{ \AA}$, $\beta = 97.566(2)^\circ$, $V = 4977.4(3) \text{ \AA}^3$, space group $P2_1/c$, $Z = 4$, $\rho = 1.309 \text{ g cm}^{-3}$, $\mu(\text{MoK}\alpha) = 5.46 \text{ cm}^{-1}$, 10374 reflections measured at 193 K in the $1\text{--}26.36^\circ$ θ range, 9886 unique ($R_{\text{int}} = 0.076$), 550 parameters refined on F^2 using 9886 reflections to final indices $R [F^2 > 4\sigma(F^2)] = 0.053$, $wR = 0.102$ [$w = 1/[\sigma^2(F_o^2) + (0.0176P)^2 + 9.0758P]$ where $P = (F_o^2 + 2F_c^2)/3$]. The final residual Fourier positive and negative peaks were equal to 0.67 and -0.63 , respectively. Crystal data were collected on a Nonius KappaCCD diffractometer at 193 K. Solution and refinement were performed using the SHELX-97 programs. CCDC 221796. See <http://www.rsc.org/suppdata/cc/b3/b312318a/> for crystallographic data in CIF or other electronic format.

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- Selected data for $(Et_4N)_2[Fe(N_2S_2Cl_2)Cl]$: Anal. Calc. For $C_{30}H_{54}Cl_3FeN_4O_2S_2 \cdot 2.5H_2O$: C 46.54, H 7.68, N 7.24, found C 46.67, H 7.62, N 6.97%. UV-vis (CH_3CN): λ/nm ($\epsilon/M^{-1} \text{ cm}^{-1}$) 365 (3514), 504 (3516). 1H NMR (250 MHz, $CDCl_3$): δ/ppm 18.5 (6H), 12 (2H), -33.2 (6H). EPR (CH_3CN , 20 K, 9.45 GHz): 4.58, 1.96. Selected IR bands (KBr pellet, cm^{-1}): ν_{CO} 1590.
- Selected data for $(Et_4N)_2[Fe(N_2S_2Cl_2)(CN)_2]$: Anal. Calc. For $C_{31}H_{54}Cl_3FeN_5O_2S_2 \cdot 2H_2O \cdot 2CH_3OH$: C 48.35, H 8.11, N 8.54, found C 48.36, H 8.10, N 8.48%. UV-vis (CH_3CN): λ/nm ($\epsilon/M^{-1} \text{ cm}^{-1}$): 360 (4972), 480 (3304). EPR (CH_3CN , 20 K, 9.45 GHz): 4.38, 2.5, 1.97. Selected IR bands (KBr pellet, cm^{-1}): ν_{CN} : 2102, ν_{CO} : 1591.
- Selected data for $(Et_4N)_3[Fe(N_2Cl_2\{SO_2\}_2)(CN)_2]$: Anal. Calc. For $C_{40}H_{74}Cl_2FeN_7O_6S_2 \cdot 2H_2O$: C 49.23, H 8.06, N 10.05, found C 49.03, H 8.06, N 10.05%. UV-vis (CH_3CN): λ/nm ($\epsilon/M^{-1} \text{ cm}^{-1}$): 404 (1263), 754 (1098). 1H NMR (250 MHz, $CDCl_3$): δ/ppm -4.1 (12H), -2.2 (2H). EPR (CH_3CN , 20 K, 9.45 GHz): 2.14, 1.96. Selected IR bands (KBr pellet, cm^{-1}): ν_{CN} : 2104, ν_{CO} : 1574, ν_{SO_2} : 1166 and 1050.
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