

Spectroscopic and crystallographic evidence for the N-protonated Fe^IFe^I azadithiolate complex related to the active site of Fe-only hydrogenases†

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The complex $[(\mu\text{-SCH}_2)_2\text{N}(\text{CH}_2\text{C}_6\text{H}_4\text{-2-Br})\text{Fe}_2(\text{CO})_6]$ and its N-protonated species, as structural models of the Fe-only hydrogenase active site, were identified spectroscopically and crystallographically, and their molecular structures show the 0.04–0.1 Å lengthening of the three N–C bonds and an intramolecular H \cdots Br contact (2.82 Å) in the crystalline state of the N-protonated species.

Since high-quality structures of the Fe-only hydrogenases isolated from *Desulfovibrio desulfuricans* and *Clostridium pasteurianum* have been revealed,^{1,2} the structural and functional models of the Fe-only hydrogenase active site (H-cluster) have turned out to be an alluring topic. The latest spectroscopic, crystallographic and theoretical studies suggest that the tether between the two iron atoms of the H-cluster is $\text{-SCH}_2\text{NHCH}_2\text{S-}$.^{3,4} Density functional calculations show that the proposed pathway for the heterolytic cleavage or formation of H₂ *via* the protonation of the bridging-N atom may provide a kinetically and thermodynamically favorable route for the Fe-only hydrogenase active site.⁵ A specially attractive issue is the potential function of the bridging-N atom as a suitable base to extract and transfer protons in the process of enzymatic H₂-evolution.⁶

The diiron azadithiolate model complexes and their reactivity have been hitherto less reported in the literature despite the putative significance of the bridging-N in the H-cluster.^{7–11} Although the protonated species of Fe^IFe^I propanedithiolate complexes have been well studied in recent years,^{12–15} efforts to isolate the N-protonated form of the Fe^IFe^I azadithiolate model complex have been unsuccessful so far.^{16,17} To stabilize the N-protonated species, we designed the Fe^IFe^I azadithiolate complex with an *ortho*-bromobenzyl group, $[(\mu\text{-SCH}_2)_2\text{N}(\text{CH}_2\text{C}_6\text{H}_4\text{-2-Br})\text{Fe}_2(\text{CO})_6]$ (**1**), the N-protonated species of which is indeed more stable than the N-protonated *para*-bromo analogue. The dithiolate complex $[(\mu\text{-SCH}_2)_2\text{NH}(\text{CH}_2\text{C}_6\text{H}_4\text{-2-Br})\text{Fe}_2(\text{CO})_6]^+\text{ClO}_4^-$ [**1(NH)**], as a model of the N-protonated Fe-only hydrogenase active site, was successfully isolated and crystallographically identified for the first time.

Complex **1** was prepared in moderate yield by treatment of Li₂Fe₂S₂(CO)₆ with (ClCH₂)₂NCH₂C₆H₄-2-Br in THF.^{7,18}

Protonation of **1** in CHCl₃ with an excess of aqueous HClO₄ solution afforded an orange precipitate [**1(NH)**] in good yield; it was washed with hexane and recrystallized in CH₂Cl₂–CH₃CN. Complex **1** is relatively stable in the solid state and in O₂-free solution, while its N-protonated form is gradually deprotonated in neutral solution.

Complex **1** in CH₃CN solution displays three $\nu(\text{CO})$ bands at 2074, 2035, 1997 cm⁻¹ in IR spectrum [Fig. 1(a)]. As an excess of HClO₄ was added to the CH₃CN solution of **1** the $\nu(\text{CO})$ bands shifted *ca.* 17 cm⁻¹ to higher frequencies [Fig. 1(b)],^{11,16} appearing at the same wavenumbers as its corresponding N-protonated complex **1(NH)**. With the addition of 0.4 equiv. of pyridine to the CH₃CN solution of **1(NH)**, six $\nu(\text{CO})$ bands appear in the IR spectrum [Fig. 1(c)], indicative of the equilibrium between **1** and **1(NH)**. The further addition of pyridine up to one equiv. results in a shift of the $\nu(\text{CO})$ absorptions to frequencies essentially equal to those of **1** [Fig. 1(d)].

The reversible protonation and deprotonation processes of **1** and **1(NH)** are shown also by the ¹H NMR spectra (Fig. 2). As 5 equiv. of HClO₄ was added to the CD₃CN solution of **1**, the singlet of the CH₂ group of the benzyl moiety at δ 4.01 [Fig. 2(a)] shifts down field by 0.53 ppm, and the singlet of the CH₂S groups at δ 3.73 splits into two broad signals at δ 3.37 and 4.19 [Fig. 2(b)].

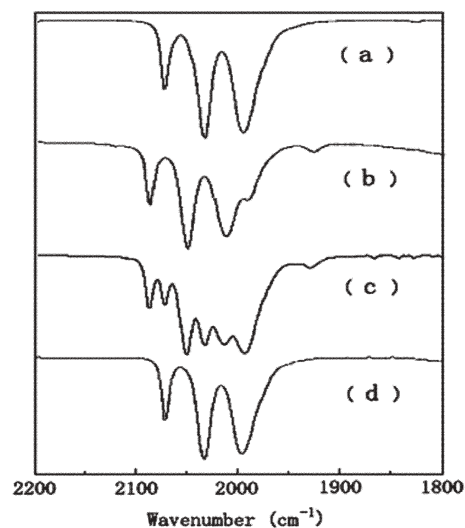


Fig. 1 FT-IR spectra of (a) **1** in CH₃CN, (b) **1** + 10 equiv. of HClO₄ in CH₃CN, (c) **1(NH)** + 0.4 equiv. of pyridine in CH₃CN, (d) **1(NH)** + 1 equiv. of pyridine in CH₃CN.

† Electronic Supplementary Information (ESI) available: Experimental details and selected bond lengths and angles for **1** and **1(NH)**. See <http://www.rsc.org/suppdata/cc/b5/b503371c/>
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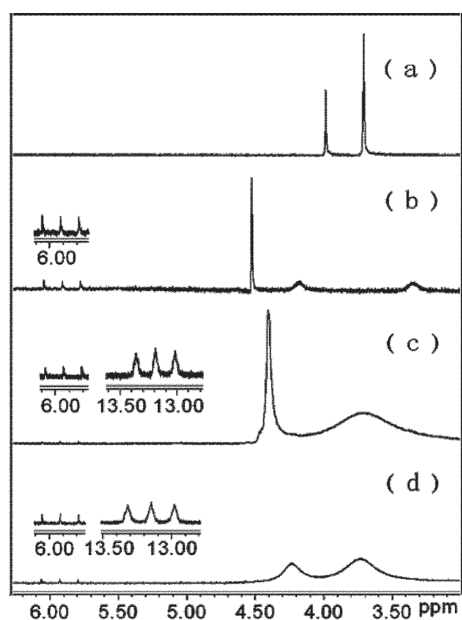


Fig. 2 ^1H NMR spectra of (a) **1** in CD_3CN , (b) **1(NH)** in CD_3CN , (c) **1(NH)** + 0.2 equiv. pyridine in CD_3CN , (d) **1(NH)** + 0.4 equiv. pyridine in CD_3CN .

In addition, a triplet with $^1J_{\text{NH}} = 53$ Hz appears at δ 5.92, attributed to the proton of the NH group of **1(NH)**. The splitting and broadening of the CH_2S resonance in the ^1H NMR spectrum of **1(NH)** indicates that after N-protonation of **1** the folding of the $\text{FeS}_2\text{C}_2\text{N}$ ring is slowed down. The two protons, which are on the equatorial bonds relative to the 6-membered ring [$\text{Fe}(1)\text{S}(2)\text{C}(15)\text{N}(1)\text{C}(14)\text{S}(1)$, Fig. 3] and *cis* to the proton of the NH group, should display higher resonances than the ones on the vertical bonds and *trans* to the proton of the NH group. All observations in the ^1H NMR spectra are consistent with the protonation of the bridging-N atom. No signal is detected in the high field region (-10 to -20 ppm), which confirms that protonation does not occur at the Fe–Fe site.^{12,15}

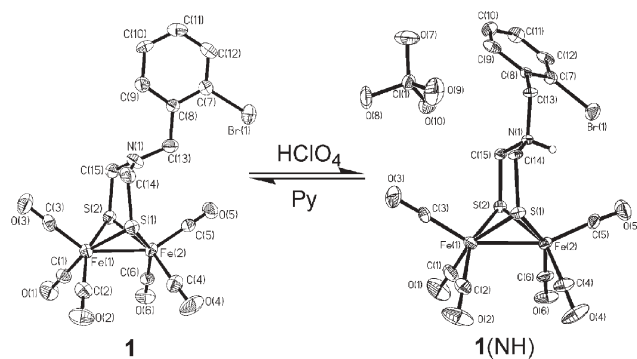


Fig. 3 Protonation and deprotonation processes and molecular structures of **1** and **1(NH)** with thermal ellipsoid set at 30% probability level. Selected distances (\AA) and angles ($^\circ$) for **1**: Fe–Fe, 2.5330(10); Fe–S, 2.2518(13)–2.2652(14); Fe–C, 1.781(6)–1.806(6); S–C, 1.863(5)–1.866(5); N–C, 1.416(6)–1.462(6); C(14)–N–C(15), 117.8(4); C(14)–N–C(13), 118.3(4); C(15)–N–C(13), 121.7(4); for **1(NH)**: Fe–Fe, 2.498(2); Fe–S, 2.237(3)–2.257(3); Fe–C, 1.767(11)–1.831(13); S–C, 1.813(8)–1.819(7); N–C, 1.456(11)–1.542(9); C(14)–N–C(15), 113.1(6); C(14)–N–C(13), 105.8(6); C(15)–N–C(13), 113.8(6); C–N–H, 108.0.

Interestingly, with the addition of 0.2 equiv. of pyridine to the CD_3CN solution of **1(NH)**, a triplet with $^1J_{\text{NH}} = 68$ Hz emerges at δ 13.15, accompanied by a decrease in the intensity of the triplet at δ 5.92. The newly-formed triplet in the low field region of the ^1H NMR spectrum is attributed to the NH of protonated pyridine.¹⁹ At the same time the two bumps of the CH_2S groups are combined into one broad signal [Fig. 2(c)]. The singlet arising from the CH_2 of the benzyl group is broadened and shifted to higher field by 0.28 ppm on addition of a further 0.2 equiv. of pyridine [Fig. 2(d)]. The subsequent addition of pyridine up to 1 equiv. changes the two broad signals of the CH_2 groups into two sharp singlets and all signals move back to the original chemical shifts of **1**. Figs. 2(c) and 2(d) prove that in the presence of pyridine the deprotonation of **1(NH)** occurs instantly to afford **1** and pyridinium perchlorate (Fig. 3).

To obtain unambiguous evidences for the N-protonation of **1**, the molecular structures of **1** and its protonated form **1(NH)** were determined by X-ray analyses of single crystals (Fig. 3 \ddagger).¹⁸ The central $2\text{Fe}_2\text{S}$ structures of **1** and **1(NH)** are both in the butterfly framework and each Fe atom is coordinated in the familiar pseudo-pyramidal geometry as in previously reported $2\text{Fe}_2\text{S}$ models.^{8–11} Although the structures of the $\text{Fe}_2\text{S}_2(\text{CO})_6$ moieties of **1** and its *para*-bromo analogue are quite similar,²⁰ the tertiary amine moiety of **1** features an apparently different conformation from its *para*-bromo analogue, probably resulting from the steric hindrance of the *ortho*-bromo substituent. The sum of the angles at the bridging-N of **1** is *ca.* 358° , suggesting an sp^2 -hybridized N atom, while it is 341° for the *para*-bromo analogue,^{20,21} roughly consistent with an sp^3 -hybridization of the N atom.

A ready comparison of the structures of **1** and **1(NH)** shows conspicuous discrepancies in the following aspects. The C(5)Fe(2)Fe(1) [$156.2(3)^\circ$] of **1(NH)** is *ca.* 7° wider than its corresponding C(3)Fe(1)Fe(2) angle [$149.4(3)^\circ$], and both angles are enlarged by $6\text{--}7^\circ$ as compared with that of **1**, bringing relief of the spatial congestion between the *ortho*-Br, apical CO and NH groups. The N-protonation of **1** results in an elongation of the three N–C bonds by $0.04\text{--}0.1$ \AA , as anticipated. It is noticeable that the *ortho*-bromobenzyl group of **1** resides in a quasi-vertical position relative to the $\text{Fe}(1)\text{S}(2)\text{C}(15)\text{N}(1)\text{C}(14)\text{S}(1)$ ring (Fig. 3), while the benzyl group stands in an equatorial position in **1(NH)**. In the light of the C–N–C angles, $117.8(4)\text{--}121.7(4)^\circ$ for **1** and $105.8(6)\text{--}113.8(6)^\circ$ for **1(NH)**, the N atom of **1(NH)** possesses a distorted tetrahedral conformation, which is in contrast to the pseudo-triangular conformation at the N atom of **1**. The other considerable discrepancy is that as the bridging-N of **1** is protonated the benzene ring of **1(NH)** rotates towards the C(3)O(3) ligand and is angled backwards. The proper $\text{N}\cdots\text{Br}$ distance [$3.334(7)$ \AA] for intramolecular $\text{N}\cdots\text{H}\cdots\text{Br}$ interactions may potentially promote the stability of the N-protonated species.¹⁸

In summary, we have successfully isolated and characterized the first N-protonated $\text{Fe}^{\text{I}}\text{Fe}^{\text{I}}$ azadithiolate model complex of the Fe-only hydrogenase active site by introduction of an *ortho*-bromobenzyl group to the bridging-N atom. Protonation and deprotonation processes between the azadithiolate complex and its N-protonated form are instant and reversible. The results of this work give experimental support to the argument that the bridging-N atom of the Fe-only hydrogenase active site may perform a

function as the proton capturer and carrier in the enzymatic H₂-production process.

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Notes and references

‡ Crystal data for **1**: C₁₅H₁₀BrFe₂NO₆S₂, *M* = 555.97, orthorhombic, space group *Pbca*, *a* = 13.3549(9), *b* = 14.8313(10), *c* = 19.7845(11) Å, *V* = 3918.7(4) Å³, *T* = 293 K, *Z* = 8, *μ* = 3.764 mm⁻¹, 27109 reflections measured, 4888 unique (*R*_{int} = 0.1567) which were used in all calculations. The final *wR*(*F*²) was 0.0793 (all data). CCDC 244661. Crystal data for **1**(NH): C₁₅H₁₁BrClFe₂NO₁₀S₂, *M* = 656.43, monoclinic, space group *P2*(1)/*c*, *a* = 22.953(10), *b* = 6.611(3), *c* = 15.033 (6) Å, *β* = 104.63(3)°, *V* = 2207.2(16) Å³, *T* = 293 K, *Z* = 4, *μ* = 3.488 mm⁻¹, 14151 reflections measured, 5515 unique (*R*_{int} = 0.2544) which were used in all calculations. The final *wR*(*F*²) was 0.1947 (all data). The high values of *R*(int) for **1** and **1**(NH) may be caused by the reason that the crystals were slightly strained by partial relaxation of the twin coherence. CCDC 258455. See <http://www.rsc.org/suppdata/cc/b5/b503371c/> for crystallographic data in CIF format.

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