Iron hydrogenase active site mimic holding a proton and a hydride

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The first model of the iron hydrogenase active site has been prepared which concomitantly carries a proton and a hydride; the title species was characterized by IR and NMR spectroscopy and is reduced at more positive potential than any other mimic of this kind.

Iron hydrogenases (Fe-H_2ase) are enzymes which catalyze the reversible reaction of protons and electrons to molecular hydrogen.[1] Whereas the crystal structure elucidation of two Fe-H_2ases provided a detailed structural picture of their active sites (H-cluster, Chart 1a),[2,3] small synthetic model complexes are valuable aids to compare and to understand the spectroscopic signatures of the enzyme.[4–7] Furthermore, functional studies on the model complexes have contributed to the understanding of the enzyme's mechanism.[8–10]

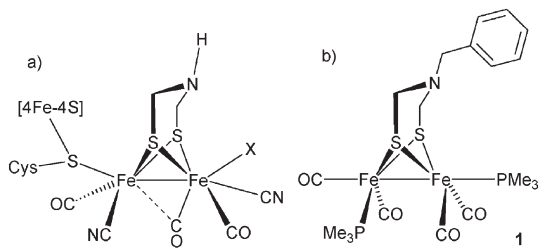

Chart 1

The active site is comprised of two unusual low-valent diiron nuclei which are coordinated by a cysteine-linked [Fe_4S_4] cluster, carbon monoxide and cyanide ligands and by a non-proteic azadithiolate (adt = S-CH_2-NH-CH_2-S) bridging the two iron centers.[11,12] As the reduction of protons or the oxidation of hydrogen follows an ionic mechanism, the H-cluster inevitably has to pass through a state where it carries a proton and a hydride. Using computational techniques, the central nitrogen atom of the dithiolate bridge was identified as a potential basic site,[13] whereas the cyanide ligands increase the electron density at the iron centers and facilitate the formation of a hydride at this position. Although the hydride has to reside at least transiently in a terminal position to engage in an intramolecular reaction with the proton,[14,15] a bridging hydride cannot be excluded as an intermediate state of the Fe-H_2ase. In fact, a bridging hydride has recently been identified in the functionally related Ni-Fe H_2ase.[16] We therefore set out to synthesize a novel Fe-H_2ase active site mimic which contains electron donating ligands at the diiron core as well as an adt linker (Chart 1b), anticipating that an unprecedented species carrying a hydride at the diiron core and a proton at the adt nitrogen may be realized. Such a structure can be regarded as a snapshot of the H-cluster just before the formation of the H–H bond.

Hence, a solution of readily synthesized [Fe_2(μ-adt)$(CO)_6$] **2** [17–19] in hexane was treated with trimethylphosphine to afford complex **1** in excellent yield.[20] Complex **1** is rather air sensitive in solution and attempts to purify the complex by column chromatography resulted in complete degradation of the product. However, addition of a little toluene and removal of the solvents and of excess trimethylphosphine at high vacuum spontaneously produced crystalline material suitable for X-ray analysis.† Single-crystal X-ray diffraction analysis of **1** (Fig. 1) reveals the usual edge-bridged bi-square-pyramidal geometry around the iron centers which are at a distance of 2.55 Å.[21] The two trimethylphosphine ligands prefer an unsymmetric geometry around the iron centers in the solid state with one in an apical and the other in a basal position. This conformation is similar to that preferred by an ethyldithiolate-bridged diiron complex where a minimum of steric hindrance at the bridge has been suggested to facilitate this arrangement.[20]

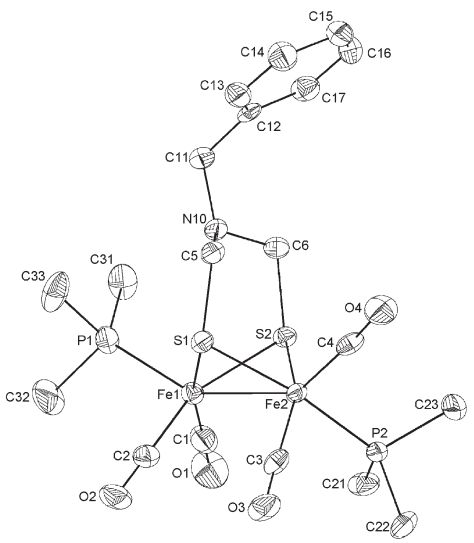

Fig. 1 ORTEP view (ellipsoids at 50% probability level) of [Fe_2(μ-$SCH_2N(CH_2Ph)CH_2S$)$(CO)_4(PMe_3)_2$] (**1**). Selected bond lengths (Å): Fe1–Fe2 2.5461(12), Fe1–S1 2.2663(18), Fe1–S2 2.2546(16), Fe2–S1 2.2560(17), Fe2–S2 2.2465(16), Fe1–P1 2.2174(18), Fe2–P2 2.2255(18), Fe1–C1 1.724(9), Fe1–C2 1.766(6), Fe2–C3 1.767(9), Fe2–C4 1.774(7).

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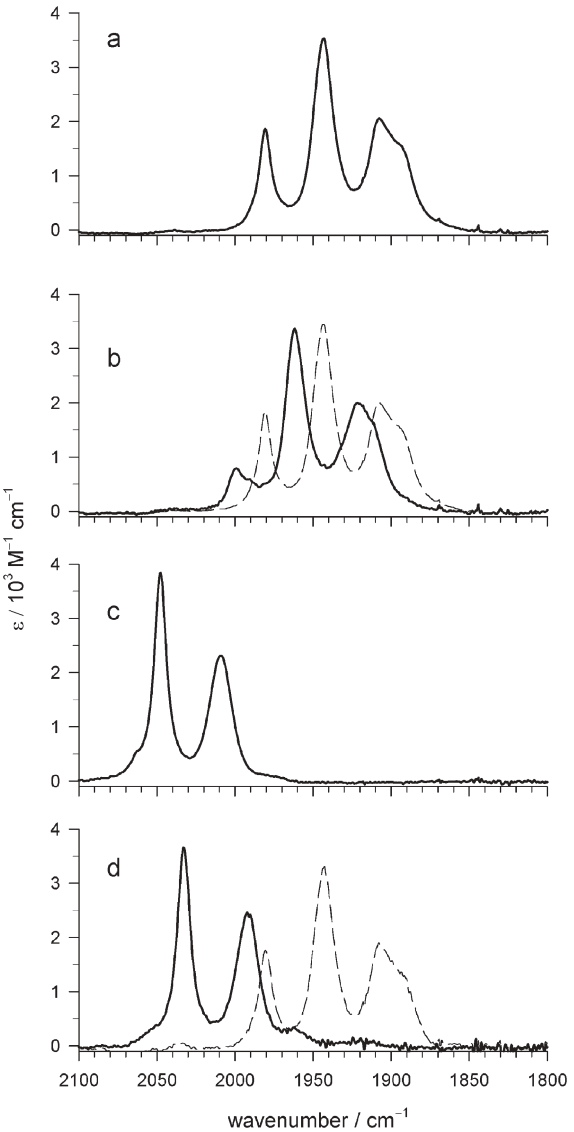
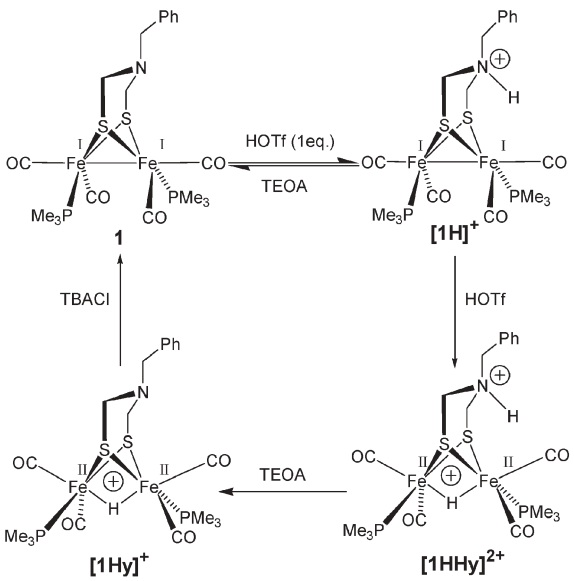


Fig. 2 Carbonyl region of the IR spectra obtained for solutions of **1** (3 mM) in CH_3CN. Protonated states were generated under the conditions indicated in parentheses. a) **1**. b) **[1H]**$^+$ (3 mM HOTf, —); Spectrum after deprotonation with triethanolamine (TEOA, ---). c) **[1HHy]**$^{2+}$ (0.2 M HOTf). d) **[1Hy]**$^+$ (0.2 M HOTf followed by TEOA, —); Spectrum after deprotonation with tetrabutylammonium chloride (TBACl, ---).

Addition of one equivalent of triflic acid to a solution of **1** in CH_3CN results in the formation of **[1H]**$^+$, as evidenced by the IR spectra (Fig. 2a, b) that show a shift of $\tilde{\nu}_{CO}$ = 16 cm^{-1} towards higher energy. This shift is typical for a protonation of the adt nitrogen.[8,17] Addition of triethanolamine (TEOA) reverses the adt protonation and complex **1** is quantitatively recovered. Upon addition of an excess of triflic acid, **[1H]**$^+$ is transformed into a product characterized by the IR spectrum shown in Fig. 2c. This spectrum can be assigned to the doubly protonated species $[Fe_2(\mu\text{-Hadt})(\mu\text{-H})(CO)_4(PMe_3)_2]^{2+}$, **[1HHy]**$^{2+}$, with an additional average shift of $\tilde{\nu}_{CO} \approx 80$ cm^{-1}, reflecting the substantial decrease of electron density at the diiron site as formally expressed by the Fe^{II}–Fe^{II} valence state of the hydride complex.[10,22] **[1HHy]**$^{2+}$ is the first Fe-H_2ase active site mimic which carries a proton at the adt nitrogen as well as a hydride at the diiron core. Initial attempts to deprotonate **[1HHy]**$^{2+}$ to recover **1** with an excess of triethanolamine failed and a new spectrum emerged which in shape resembled that of **[1HHy]**$^{2+}$, however shifted by $\tilde{\nu}_{CO} \approx$ 16 cm^{-1} towards lower frequencies. It thus seems that the adt nitrogen is selectively deprotonated, leaving the hydride portion intact and the species formed is identified as the hydride complex $[Fe_2(\mu\text{-adt})(\mu\text{-H})(CO)_4(PMe_3)_2]^+$, **[1Hy]**$^+$. The formation of **[1Hy]**$^+$ by deprotonation of **[1HHy]**$^{2+}$ relies on the inertness of the hydride towards nitrogen bases. The sluggish deprotonation behavior with nitrogen bases has been reported for a related hydridic diiron complex,[10] but could be overcome by the addition of cyanide or chloride.[23] In the case of **[1Hy]**$^+$ addition of tetrabutylammonium chloride results in quantitative recovery of **1** (Fig. 2d, dashed line). Complex **1** constitutes a dibasic species which, as such, should give rise to *three* different protonation states. However, owing to the remarkable difference in deprotonation kinetics between the adt nitrogen and the Fe–Fe bond, complex **1** can selectively be prepared in *four* protonation states, **1**, **[1H]**$^+$, **[1Hy]**$^+$, and **[1HHy]**$^{2+}$ (Scheme 1).[24]

NMR characterization of all four states shows that protonation of the adt nitrogen renders the two $Fe(CO)_2P(CH_3)_3$ subunits in **[1H]**$^+$ and **[1HHy]**$^{2+}$ non-equivalent as the ring inversion of the six-membered metalloazaheterocycle is restricted. Hence, the trimethylphosphine protons which give rise to one doublet in the ^{1}H NMR spectra of **1** and **[1Hy]**$^+$ due to their coupling to the phosphorus center, are split into two doublets at δ = 1.56, 1.54 ppm for **[1H]**$^+$ and at δ = 1.57, 1.63 ppm for **[1HHy]**$^{2+}$. Similarly, the ^{31}P NMR spectra of the adt-protonated **[1H]**$^+$ and **[1HHy]**$^{2+}$ feature two phosphorus signals at δ = 31.1, 19.8 ppm for **[1H]**$^+$ and at δ = 25.3, 21.3 ppm for **[1HHy]**$^{2+}$ whereas only one signal is observed in the spectra of **1** and **[1Hy]**$^+$ (Fig. 3). The hydride is visible in the ^{1}H NMR spectra of **[1Hy]**$^+$ and **[1HHy]**$^{2+}$ as a triplet ($J_{H\text{-}P}$ = 22 Hz) at δ = −15.6 ppm and as a doublet of doublets ($J_{H\text{-}P}$ = 21, 23 Hz) at similar chemical shift, respectively. Since the same coupling constant can be observed in the ^{31}P NMR of the respective



Scheme 1 Protonation reactions of **1** in acetonitrile solution with triflic acid (HOTf). Deprotonation of the adt nitrogen with triethanolamine (TEOA) and of the hydride with tetrabutylammonium chloride (TBACl).

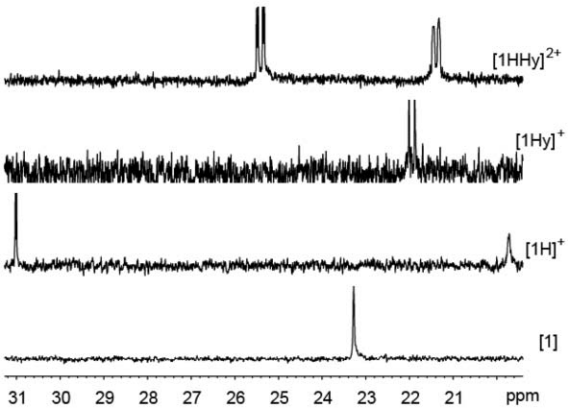


Fig. 3 ^{31}P NMR spectra of **1**, **[1H]**$^+$, **[1Hy]**$^+$ and **[1HHy]**$^{2+}$ in CD_3CN at 25 °C. Prepared from **1** (6 mM) under conditions similar to those used in the IR experiments.

species, it is clear that the hydride in **[1Hy]**$^+$ and **[1HHy]**$^{2+}$ resides in a bridging position[25] and that the complexes are stable even under these drastic acidic conditions. In contrast to the solid state structure, the phosphine ligands prefer a symmetrical basal position relative to the iron centers in **[1Hy]**$^+$ and **[1HHy]**$^{2+}$ as evidenced by the relatively large coupling constants of J_{H-P} = 22 Hz.[20]

Voltammetric investigation of complex **1** reveals an irreversible reduction at *ca.* −2.2 V *vs.* $Fc^{+/0}$ and the potential for the first reduction is shifted to −1.0 V for **[1HHy]**$^{2+}$. This dramatic shift of 1.2 V is a result of the fact that **[1HHy]**$^{2+}$ concurrently carries a proton and a hydride.[26]

In summary, we have synthesized the first biomimetic model of the Fe-H_2ase active site which can be protonated on either the Fe–Fe bond or the adt nitrogen as well as on both sites simultaneously. All four protonation states are well-defined and have been characterized by IR and NMR spectroscopy. Double protonated **[1HHy]**$^{2+}$ is reduced at −1.0 V *vs.* $Fc^{+/0}$, a potential considerably more positive than that of any other Fe-H_2ase mimic ever reported. Reduction at such mild potential is a result of the two preceding protonations, one of which involves the adt nitrogen.[26] From a structural point of view, **[1HHy]**$^{2+}$ resembles an intermediate closer to the hydrogen formation event than other model complexes which only carry either a hydride or a proton.[8,10] With regard to the enzyme, the question arises as to whether the catalytic cycle involves the formation of a double protonated species at the Fe^I–Fe^I level similar to our model system.

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Notes and references

† **[Fe_2(μ-$SCH_2N(CH_2Ph)CH_2S$)$(CO)_4(PMe_3)_2$]** (**1**). Trimethylphosphine (204 mg, 2.68 mmol) was added to a solution of complex **2** (160 mg, 0.335 mmol) in 5 ml hexane under nitrogen atmosphere. After 3 h of stirring, the solvent and unreacted trimethylphosphine were removed *in vacuo*. The resulting red-brown solid was re-dissolved in toluene and the solution was filtered through a plug of Celite. The solvent was removed, and the deep red solid was washed with cold hexane (174 mg, 91%). Single crystals suitable for X-ray analysis were obtained from toluene/hexane solution upon rapid concentration *in vacuo*. Anal. calculated for $C_{19}H_{29}Fe_2NO_4P_2S_2$: C, 39.81; H, 5.10; N, 2.44. Found: C, 39.83; H, 5.27; N, 2.38%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30–7.26 (m, 3H, Ar*H*), 7.18 (d, J = 6.8 Hz, 2H, Ar*H*), 3.55 (s, 2H, NCH_2Ph), 3.08 (s, 4H, SCH_2N), 1.50 (d, J = 9.2 Hz, 18H, PMe_3). ^{13}C NMR (100.6 MHz, THF-d_8): δ = 217.5, 217.3, 137.9, 130.3, 129.1, 128.3, 63.8, 54.2, 20.8, 20.4 (2C). ^{31}P NMR (161.9 MHz, CD_3CN): δ = 23.3. IR (CH_3CN, cm^{-1}): ν_{CO} = 1980, 1943, 1907, 1892 (sh). Crystallographic data of **1**: Monoclinic, a = 10.7622(14) Å, b = 19.773(3) Å, c = 11.7983(15) Å, β = 90.390(15)°, vol = 2510.7(6) Å^3, T = 293(2) K, $P2_1/c$, Z = 4, μ = 1.474 mm^{-1}, $N_{measured}$ = 16225, $N_{unique,\ all\ data}$ = 4834, $N_{unique,\ (I \geqslant 2\sigma(I))}$ = 3035, R_{int} = 0.1047, $wR2$ = 0.1650 (all data), $R1$ = 0.0492 ($I \geqslant 2\sigma(I)$). CCDC 283269. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b514280f

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21 CCDC 283269 (**1**) and CCDC 283270 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center *via* www.ccdc.cam.ac.uk/data_request/cif.
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24 Since no interconversion between **[1H]**$^+$ and **[1Hy]**$^+$ could be observed on the timescale of hours, it cannot readily be determined which mono-protonated species is the thermodynamically stable form.
25 In addition, two-dimensional ^{1}H-^{31}P correlation spectroscopy (HMQC) of **[1HHy]**$^{2+}$ revealed the coupling of the hydride to both phosphorus centers.
26 Reference 10 describes a related diiron-hydride complex lacking the adt nitrogen which can be protonated at a cyanide ligand instead. This species, however, is reduced at −1 V *vs.* Ag/AgCl, corresponding to less than −1.4 V *vs.* $Fc^{+/0}$.

