The iron centre of the cluster-free hydrogenase (Hmd): low-spin Fe(II) or low-spin Fe(0)?†

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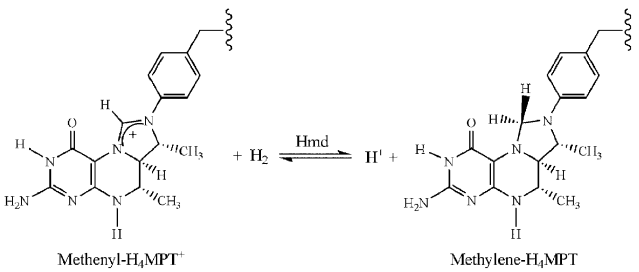
Received (in Cambridge, UK) 28th March 2008, Accepted 29th April 2008
First published as an Advance Article on the web 4th June 2008
DOI: 10.1039/b805262j

Infrared data for mono-iron complexes possessing two *cis*-CO together with Mössbauer data for the enzyme and a model complex support the assignment that the iron centre of the cluster-free hydrogenase Hmd is low-spin Fe(II).

The [Fe]-hydrogenase, H_2-forming methylenetetrahydromethanopterin dehydrogenase (Hmd), the sometimes called iron–sulfur cluster free hydrogenase, is one of the three phylogenically unrelated hydrogenases.[1] The other two are [FeFe]- and [NiFe]-hydrogenases, which catalyse reversibly and rapidly hydrogen evolution.[2] Unlike the [FeFe]- and [NiFe]-hydrogenases, this particular enzyme catalyses reversibly a hydrogenation reaction *via* a specific substrate, N^5,N^{10}-methenyltetrahydromethanopterin, methenyl-H_4MPT^+, Scheme 1.[3,4]

Early investigations using a variety of spectroscopic techniques show that the iron centre of the co-factor is low-spin, either Fe(II) or Fe(0), around which two *cis*-CO and one pyridone derivative are intrinsic ligands.[4,5] More recently the crystal structure of the enzyme was solved at 1.75 Å resolution by Thauer and co-workers.[6] The crystal structure shows that the iron centre takes a square pyramidal geometry in which the sp^2-hybridised N of the pyridone derivative binds apically to the iron and two *cis*-CO, with a cysteinyl thiolate and an unknown ligand occupying the basal positions. Within a hydrogen bonding distance to the iron *trans* to the pyridone derivative there is one water molecule, Fig. 1(a). The oxidation state of the iron centre remains however undefined. Without knowledge of the net charge on an enzymic metallo-centre which is not available from the crystallography, metal atom oxidation states cannot be confidently assigned. This problem has and continues to bedevil the assignment of oxidation states



Scheme 1 The reversible hydrogenation reaction catalysed by Hmd.

for FeMoco, the active site of molybdenum nitrogenase[7] and indeed early work on [FeFe]-hydrogenase.[8] In the latter case spectroscopic studies of synthetic complexes which modelled the subsite of the enzyme led support to the unprecedented occurrence of Fe(I) systems in biology.[8–12]

In this paper we describe the synthesis of a mono-iron complex possessing two *cis*-CO, N-pyridine and thiolate ligation which has some structural relevance to the Hmd and which provides insight with respect to the oxidation state of the iron in the isolated co-factor.[13]

To this end we have utilised the known multidentate ligand 2,2′-(pyridin-2-ylmethylazanediyl)diethanethiol, $\mathbf{H_2L}$. It has ligating atoms in common with those found in the co-factor, *viz*. a $\{N_2S_2\}$ donor-set. It was prepared by the reaction of 2-aminomethylpyridine with ethylene sulfide in toluene (Scheme 2),[14] and is known as an effective tridentate chelating ligand, forming complexes with the $\{Re(fac\text{-}CO)_3\}^+$ core for

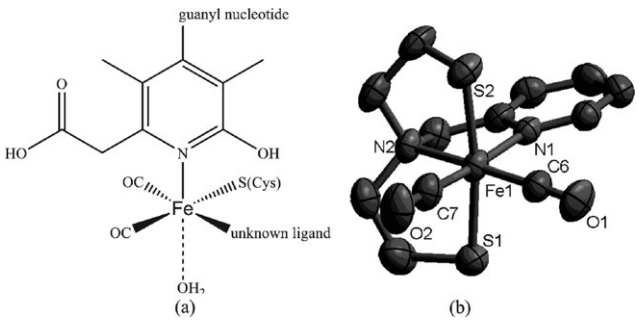


Fig. 1 (a) Schematic view of the iron centre of the co-factor[6] and (b) The crystal structure of the model complex (the thermal ellipsoids are drawn at 50% probability level and hydrogen atoms, for clarity, are omitted), [Fe(*cis*-CO)$_2$**L**], Fe1–N1 2.005, Fe1–N2 2.039, Fe–C6 1.751, Fe–C7 1.755, Fe1–S2 2.3089, Fe1–S1 2.3067; S2–Fe1–S1 171.97, C7–Fe1–N1 175.95, C6–Fe1–N2 174.26, N1–Fe1–N2 82.75, C6–Fe1–C7 89.08.

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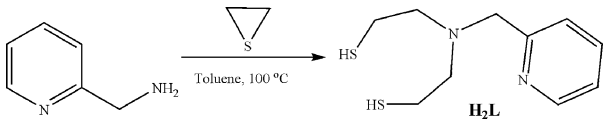
† Electronic supplementary information (ESI) available: Experimental procedures, Mössbauer spectra, electrochemistry, UV/Vis spectrum and crystal data and structural refinement. CCDC 683069. For ESI and crystallographic data in CIF or other electronic format see DOI:10.1039/b805262j



Scheme 2 Synthetic route of the pyridine-based ligand, H_2**L**.

example.[15] It also readily forms complexes with high valent transition metals (Mo and W) with two *cis*-oxo ligands.[16]

The reaction of this H_2**L** with $FeSO_4 \cdot 7H_2O$ proceeds within minutes in methanol at room temperature to give under carbon monoxide a dark-red solution from which dark-red crystal blocks were obtained on cooling (−25 °C under CO atmosphere for two days).‡ These were suitable for single crystal X-ray analysis.

The crystal structure of the mono-iron complex, [Fe(*cis*-$CO)_2$**L**] together with selected bond lengths (Å) and angles (°) is shown in Fig. 1(b).§ The iron centre takes a slightly distorted octahedral geometry in which the iron atom lies slightly out of the plane composed by {N1–N2–C6–C7} atoms towards the S1 atom. The two CO ligands at the equatorial positions are *cis* to each other with a nearly perfect right angle. Due to the constraints imposed by the chelating rings constituted by the coordination of the {S2N2S1} donor atoms with the iron, the axis along the {S2–Fe1–S1} bonds is slightly bent. Similar constraint originating from the chelating ligation of N1 and N2 atoms to the metal centre leads to a comparatively large deviation of the bond angle of N1–Fe1–N2 from 90°.

The neutrality of the isolated complex indicates that the oxidation state of the iron centre does not change during the reaction. Mössbauer spectroscopic analysis for the solid complex at 80 K (Fig. S1, ESI†) gives 0.10 and 0.79 mm s^{-1} for the isomer shift (i.s.) and quadrupole splitting (q.s.) parameters, respectively. The small i.s. and temperature independent q.s. are consistent with low-spin Fe(II).[17,18] These values are comparable to those of the native enzyme (0.06 and 0.65 mm s^{-1} at 80 K) and the extracted co-factor (0.03 and 0.43 mm s^{-1} at 80 K) in frozen solution.[4]

The infrared spectrum of the complex is shown in Fig. 2 (bottom), which possesses the characteristic spectral pattern of transition metal complexes with two *cis*-CO.[19] It is strikingly close to that of the co-factor isolated from the enzyme, Fig. 2 (top).[5] The average of the two absorption bands for the

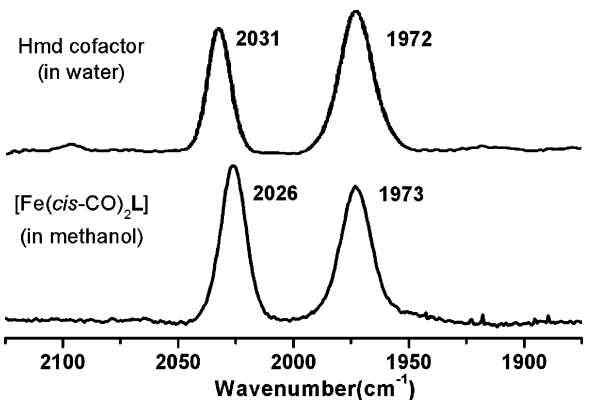


Fig. 2 Infrared spectra of the co-factor[5] and the complex [Fe(*cis*-$CO)_2$**L**].

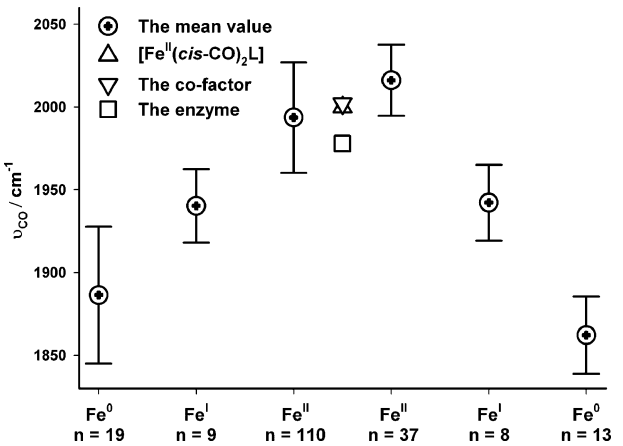


Fig. 3 The correlations between the average of the IR absorption bands and the oxidation state of the iron in monoiron complexes with {Fe(*cis*-$CO)_2$} core (*n* is the number of IR data used in the plot).

complex is only 2 cm^{-1} lower than that for the isolated co-factor.

The oxidation state of the synthetic complex is unambiguously Fe(II) but before asserting that of the cofactor is the same we need to understand how tight a correlation exists between Fe oxidation states and the infrared of the complexes possessing the {Fe(*cis*-$CO)_2$} core. To this end we have plotted the averages of the two infrared absorption bands for all complexes documented in the literature against the known iron oxidation state.[20–36]¶ The left hand side of the plot shown in Fig. 3 shows the evident correlation between the observed oxidation states and the averages of the IR absorption bands. There is overlapping of the band averages between the oxidation states; there is excellent linearity between the mean values and the oxidation states, ($R > 0.9999$). The right hand side of the plot is for a restricted set of ligands in which complexes with alkenyl, cyclopentadienyl and other nominally abiological organometallic groups are excluded.

For this set the mean value for each oxidation state is Fe(0): 1862 ± 23 cm^{-1}, Fe(I): 1942 ± 23 cm^{-1} and Fe(II): 2016 ± 22 cm^{-1} with the magnitude of ν_{CO} increasing by 77 ± 4.5 cm^{-1} for one unit increase in oxidation state.

Whether the full or restricted ligand set of data for the *cis* carbonyl complexes is considered, it is clearly evident that there is no overlap of ν_{CO} average for the cofactor and enzyme with Fe(0) data, Fig. 3. Since the possibility of an Fe(I) oxidation state for the cofactor and enzyme must be excluded on the grounds that these are diamagnetic EPR-silent systems, correlation of the experimental data strongly supports low-spin Fe(II) as the oxidation state for the biological system. Mössbauer spectral parameters (i.s. and q.s.) for the synthesised low-spin Fe(II) complex (Fig. S1, ESI†) are also in good agreement with those of the native enzyme and its extracted co-factor (*vide ante*):[4] this further reinforces the conclusion and supports the earlier postulate of Thauer and co-workers.[5]

We thank the Natural Science Foundation of China (Grant Number: 20571038) (X. L.), the State Key Laboratory of Coordination Chemistry at Nanjing University (China) (X. L.), the Royal Society (X. L., visiting C. J. P. at UEA, UK), the EPSRC Supergen 5 program (C. J. P.) and the Biotechnology

and Biological Science Research Council, UK (D. J. E.) for supporting this work.

Notes and references

‡ Characterisation of the complex, [Fe(*cis*-CO)$_2$**L**]: ^{1}H NMR ($CDCl_3$ under CO): 8.943 (d, *H*-3-Py, J = 4.48 Hz), 7.711 (t, *H*-4-Py, J = 6.92 Hz), 7.327 (t, ill-resolved, *H*-5-Py), 7.211 (d, *H*-6-Py, J = 7.28 Hz), 4.398 (s, CH_2-2-Py), 3.522 (d, 2CH_2, J = 5.60 Hz), 2.765 (d, CH_2, J = 11.88 Hz), 2.572 (t, ill-resolved, CH_2); microanalysis (%) for $C_{12}H_{14}N_2O_2S_2Fe$ (338.32), calc. (found): C 42.61 (42.52), H 4.17 (4.20), N 8.28 (8.03).

§ *Crystal data and structure refinement* for [Fe(*cis*-CO)$_2$**L**] ($C_{12}H_{14}N_2O_2S_2Fe$, M = 338.32, T = 273(2) K): triclinic, $P\bar{1}$, a = 7.1525(11), b = 7.6915(12), c = 13.464(2) Å, α = 91.659(2), β = 95.127(2), γ = 109.7330(10)°; V = 693.05(18) Å^3, Z = 2, Limiting indices, $-8 \le h \le 8$, $-8 \le k \le 9$, $-15 \le l \le 15$; reflections collected/unique, 4118/2205 [R_{int} = 0.0232]; Goodness-of-fit on F^2, 1.010; Final R indices [$I > 2\sigma(I)$], R_1 = 0.0446, wR_2 = 0.1207; R indices (all data), R_1 = 0.0588, wR_2 = 0.1353.

¶ Complexes with ligands employing atoms of group IIIA as ligating atoms are not included due to their retrodative bonding nature.[37] Infrared data are gathered regardless of how the data were collected.

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