H/D Exchange Reactions in Dinuclear Iron Thiolates as Activity Assay Models of Fe−H_2ase

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The diiron unit in the H-cluster of Fe-only hydrogenases, Figure 1, is unique in its precedent for low-valent organo-metallic molecules as structural and spectroscopic models.[1−4] Thus the bridged dithiolato complex **1**, (μ-pdt)$Fe_2(CO)_6$ (pdt = $SCH_2CH_2CH_2S$),[5] the bridge-modified derivatives, (μ-$SCH_2N(Me)CH_2S$)-$Fe_2(CO)_6$,[6] (μ-$SCH_2(C(H)CH_2SMe)CH_2S$)-$Fe_2(CO)_5$,[7] and dicyano derivatives such as (μ-pdt)$[Fe(CO)_2(CN)]_2^=$, complex **2**,[8] demonstrate that Fe^IFe^I dinuclear complexes match the major geometrical features of the available active site structures. The Fe−Fe distance of 2.6 Å observed in four protein crystal structures, is similar to those found for the Fe^IFe^I model complexes. Not seen in ground-state structures of the latter is the bridging or semi-bridging CO shown in Figure 1.[1,9] Infrared spectroscopic studies of enzymes derived from *Desulfovibrio desulfuricans* as well as *D. vulgaris*, indicate that the μ-CO switches to terminal in the reduced forms.[3,10] According to the crystallography, this evokes a minor structural rearrangement, retaining the short Fe−Fe distance.[3]

Iron−iron bonded moieties are extremely attractive as reactive units. DFT calculations have characterized the HOMO of complex **1** as the Fe−Fe bond density, providing a site for reactivity with electrophiles.[11,12] Herein we describe reactivity of Fe^IFe^I and $[Fe^{II}-H-Fe^{II}]^+$ complexes that is consistent with the chemical characteristics/activity of the enzymes.

Hydrogenases convert protons and electrons into H_2, reversibly, Figure 1. The active site, and functional models thereof, are thus required to take up H_2, typically assayed in enzyme activity studies by H/D exchange processes ($H_2/D_2 \rightarrow$ HD and $H_2/D_2O \rightarrow$ HD/DOH), and activate it heterolytically, (H^-/H^+).[13,14] As H_2 binding is prominent in d^6 metal complexes,[15] whereby the acidity of H_2 is greatly amplified,[16] a role for Fe^{II} in H_2 uptake and H/D exchange is suggested.

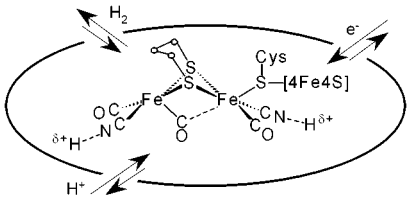
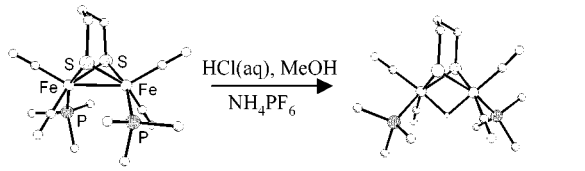


Figure 1. The active site of Fe-only hydrogenase



ν(CO) 1979, 1942, 1898 cm-1

2029, 1989 cm-1
1H NMR −15.3 t, J_{P-H} = 22 Hz

Figure 2. Structures and spectra of **3** and [**3**-H^+]PF_6^-

From the dinuclear Fe^IFe^I complexes described above as structural models, $Fe^{II}Fe^{II}$ complexes are expected to result from protonation, engaging the Fe−Fe bond density in the formation of a bridging hydride species. The all-carbonyl complex **1** is of insufficient basicity to form a stable conjugate acid (ν(CO) of (μ-pdt)$Fe_2(CO)_6$ = 2074, 2036, and 1995 cm^{-1}). However, as indicated by ν(CO) values lowered by ca. 100 cm^{-1}, the electron-rich character of the dicyano derivative **2**, allows reaction with HCl, eq 1. The subsequent positive shift of ν(CO) and ν(CN) values, as well as high field resonances in the ^{1}H NMR spectrum, are evidence that protonation has produced a bridging hydride, $[Fe^{II}-H-Fe^{II}]^+$. This reaction is complicated both by the presence of cyanide positional isomers in the reactant,[6,8] explaining the presence of two hydride resonances (−16.1 and −19.7 ppm) in the product, as well as the protonation of iron-bound cyanide, presumably producing a labile CNH ligand and overall instability.[17]

(μ-pdt)$[Fe(CO)_2(CN)]_2^= + H^+ \rightarrow$

(μ-H)(μ-pdt)$[Fe(CO)_2(CN)]_2^-$ (1)

ν(CN)	2075	2108 cm^{-1}
ν(CO)	1964, 1924, 1885	2048, 2024, 1987 cm^{-1}

In contrast, double CO substitution by PMe_3 in (μ-pdt)$Fe_2(CO)_6$, yields only one isomer as noted by NMR and by the X-ray crystal structure of (μ-pdt)$[Fe(CO)_2(PMe_3)]_2$, (**3**). Reaction with concentrated HCl yields a stable bridging hydride in the same transoid configuration, **3**-H^+, Figure 2.[18] Importantly, the differences in ν(CO) between (μ-pdt)$[Fe(CO)_2(PMe_3)]_2$ vs (μ-pdt)$[Fe(CO)_2(CN)]_2^=$, and in the product hydrides, are only 15 to 20 cm^{-1}, see eq 1 and Figure 2, suggesting similar Fe−Fe bond densities, and similar electronic characteristics of the respective bridging hydrides.

The molecular structures of complexes **3** and **3**-H^+, as its PF_6^- salt, Figure 2, are comparable, with the distinction that the Fe−Fe bond density in the former has now a proton imbedded within it. The 2Fe2S butterfly cores overlay closely. The Fe^I-Fe^I bond distance of **3** is 2.555(2) Å, while the $Fe^{II}-Fe^{II}$ distance in **3**-H^+ is 2.578(1) Å; both are faithful to the Fe- -Fe distances in the Fe−H_2ase active sites.

(1) Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science* **1998**, *282*, 1853−1858.
(2) Nicolet, Y.; Piras, C.; Legrand, P.; Hatchikian, C. E.; Fontecilla-Camps, J. C. *Structure* **1999**, *7*, 13−23.
(3) Nicolet, Y.; De Lacey, A. L.; Vernéde, X.; Fernandez, V. M.; Hatchikian, E. C.; Fontecilla-Camps, J. C. *J. Am. Chem. Soc.* **2001**, *123*, 1596−1601.
(4) Nicolet, Y.; Lemon, B. J.; Fontecilla-Camps J. C.; Peters, J. W. *Trends Biochem. Sci.* **2000**, *25*, 138−143.
(5) Lyon, E. J.; Georgakaki, I. P.; Reibenspies, J. H.; Darensbourg, M. Y. *Angew. Chem., Int. Ed.* **1999**, *38*, 3178−3180.
(6) Lawrence, J. D.; Li, H.; Rauchfuss, T. B.; Bénard, M.; Rohmer, M.-M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1768−1771.
(7) Razavet, M.; Davies, S. C.; Hughes D. L.; Pickett, C. J. *Chem. Commun.* **2001**, 847−848.
(8) Schmidt, M.; Contakes, S. M.; Rauchfuss, T. B. *J. Am. Chem. Soc.* **1999**, *121*, 9736−9737.
(9) Lemon, B. J.; Peters, J. W. *Biochemistry* **1999**, *38*, 12969−12973.
(10) Pierik, A. J.; Hulstein, M.; Hagen, W. R.; Albracht, S. P. J. *Eur. J. Biochem.* **1998**, *258*, 572−578.
(11) Lyon, E. J.; Georgakaki, I. P.; Reibenspies, J. H.; Darensbourg, M. Y. *J. Am. Chem. Soc.* **2001**, *123*, 3268−3278.
(12) Lyon, E. J. Ph.D. Dissertation, Texas A&M University, 2001.
(13) Peters, J. W. *Curr. Opin. Struct. Biol.* **1999**, *9*, 670−676.
(14) Adams, M. W. *Biochim. Biophys. Acta* **1990**, *1020*, 115−145.
(15) Kubas, G. J. *Acc. Chem. Res.* **1988**, *21*, 120−128.
(16) Abdur-Rashid, K.; Fong, T. P.; Greaves, B.; Gusev, D. G.; Hinman, J. G.; Landau, S. E.; Lough, A. J.; Morris, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 9155−9171.
(17) Fong, T. P.; Forde, C. E.; Lough, A. J.; Morris, R. H.; Rigo, P.; Rocchini, E.; Stephan, T. *J. Chem. Soc., Dalton Trans.* **1999**, 4475−4486.
(18) Savariault, J.-M.; Bonnet, J.-J.; Mathieu, R.; Galy, J. *C. R. Acad. Sci. Paris* **1977**, *284*, C 663−665.

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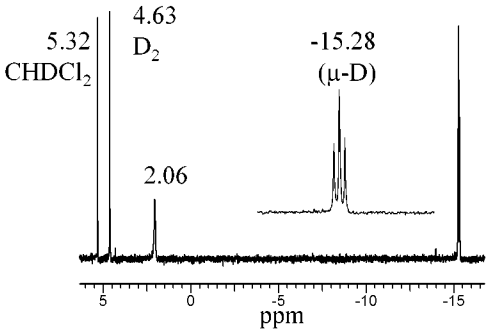


Figure 3. ^{2}H NMR spectrum of D_2-enriched [3·H^+][PF_6^-], 8 bar D_2.

Various NMR active nuclei in (μ-H)(μ-pdt)[Fe(CO)$_2$(PMe_3)]$_2^+$ PF_6^- permit study of H/D exchange processes. Following the procedure of Sellmann et al.,[19,20] medium-pressure NMR sample tubes containing **3-H**$^+$ PF_6^- dissolved in CH_2Cl_2 were pressurized with D_2 gas to 7−8 bar. After ca. 4 h at room temperature and ambient laboratory lighting, the ^{2}H NMR spectrum was measured, showing dissolved D_2 at 4.63 ppm, natural abundance $CDHCl_2$ at 5.32 ppm, and a small triplet in the high field region, −15.28 ppm, J_{P-D} = 3.32 Hz, Figure 3. The intensity of this triplet increased slowly under laboratory light and was dormant in the dark. When exposed to sunlight (Texas, July), extensive H/D exchange occurred within 3 h.

The ^{1}H NMR spectrum of the D_2/D-enriched **3-H**$^+$ PF_6^- indicated loss of intensity of the bridging hydride resonance at −15.3 ppm. The proton-decoupled ^{31}P NMR spectrum showed only resonances for the PF_6^-, the $\underline{P}Me_3$ in **3-H**$^+$ PF_6^- at 21.46 ppm, and a 1:1:1 triplet from $\underline{P}Me_3$ in **3-D**$^+$ PF_6^- at 21.54 ppm with J_{PD} of 3.32 Hz. Solutions of **3-H**$^+$ PF_6^- dissolved in CD_2Cl_2 placed under 12 bar D_2 exhibited in the ^{1}H NMR spectrum a 1:1:1 triplet centered at 4.57 ppm with J_{D-H} of 42.8 Hz indicating the presence of HD.[19,20] Catalysis in the H/D exchange from H_2/D_2 mixtures at 6 bar each and ca. 3.0% **3-H**$^+$ PF_6^- in CH_2Cl_2 was illustrated by ^{1}H NMR detection of HD in amounts $>H_2$. That there was no decomposition was indicated by the infrared spectra of D-exchanged samples which showed a ν(CO) region identical to the original samples. Pressurization with H_2 gas returned the D-exchanged product to the protio form. Under photolysis, ^{13}CO-saturated solutions of **3-H**$^+$ PF_6^- showed exchange with intrinsic ^{12}CO completely reversibly, with no apparent PMe_3 loss.

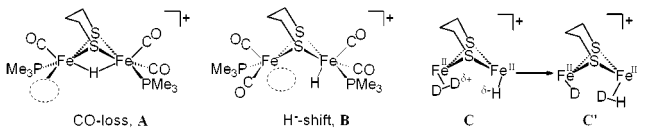
Despite these indications that the H/D exchange reaction was cleanly reversible and the coordination sphere of **3-H**$^+$ was intact, another ^{2}H signal in the range of 1.7 to 2.8 ppm emerged during the reactions in CH_2Cl_2 This resonance does not appear in acetone solutions or with triflate as counterion. Integration of the ^{1}H spectrum of samples from extensive D_2/H exchange suggests no D-exchange into PMe_3, pdt, or solvents. The possibility of some decomposition involving insolubles and $DPMe_3^+$ or RSD is under investigation.[21]

While the presence of dissolved D_2 in pressurized solutions of **3-H**$^+$ [PF_6^-] in CH_3CN was evident from the ^{2}H resonance at 4.61 ppm, there was no exchange into the bridging hydride position. In addition, CO-saturated solutions of **3-H**$^+$ [PF_6^-] in CH_2Cl_2 pressurized with D_2 showed a minor amount of the **3-D**$^+$ signal under the standard photolysis conditions (3 h sun).

Exchange of deuterium from D_2O into samples of **3-H**$^+$ [PF_6^-] in CH_2Cl_2 was not observed. However, with acetone solvent, a slow exchange occurred, which accelerated on addition of small amounts of PPN^+Cl^-. Likewise, H/D exchange with MeOD in CH_2Cl_2 required added Cl^- to serve as a proton-carrier or abstracting agent,[22] overcoming the kinetic inertness/barrier of proton transfer from **3-H**$^+$ [PF_6^-].[23]

This work illustrates that a protonated Fe−Fe bond in the dithiolate, diiron complexes which serve as structural and spectroscopic models of the Fe-only H_2ase active sites, satisfies the requirements for the enzyme-activity assays of H/D exchange from H_2/D_2 gas mixtures as well as from H_2/D_2O. Promotion of the D_2/**3-H**$^+$ H/D exchange reaction by sunlight, and its inhibition by CO imply that an open site for D_2 binding prior to D−D cleavage is a key step in the reaction path. The lack of reactivity in CH_3CN is consistent with the results of Morris et al., that CH_3CN is a better ligand for Fe^{II} than is H_2.[16]

While CO dissociation may account for the open site, structure **A**, also appealing is a hydride shift from bridging to terminal position, **B**. The binding of D_2 at the site proximal to the bridging hydride, displayed below as structure **C**, would lead to the exchange in **C′**.



A noteworthy conclusion from this suggested reaction path, is that the hydride, generated from H^+ and electrons from two Fe^I, serves as an internal base for the heterolytic cleavage of Fe^{II}-bound D_2. There is no requirement for another base to be built into this model, the suggestion of which for the Fe-only H_2ase active site[3] has guided theoretical studies[24] and synthetic programs[6] into the production of an S-to-S linker containing a central amine functionality. Should the 3-light atom S to S linker *not* be designed to provide this built-in base, we suggest its importance is in *maintaining the butterfly 2Fe2S core and optimally short Fe-* -Fe distances, throughout the $Fe^{II}Fe^{II}$ reaction process. This work also shows that the formation of a bridging or terminal hydride is a reasonable activation step in the enzymatic H_2 uptake process.

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Supporting Information Available: Molecular structures (PDF) and X-ray crystallographic tables for (μ-$SCH_2CH_2CH_2S$)[Fe(CO)$_2$$PMe_3$]$_2$, and {($\mu$-H)($\mu$-$SCH_2CH_2CH_2S$)[Fe(CO)$_2$$PMe_3$]$_2$}$^+$ PF_6^- (CIF); the synthesis and isolation of these compounds as well as a listing of control experiments for H/D exchange studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Sellmann, D.; Geipel, F.; Moll, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 561−563.
(20) Sellmann, D.; Fürsattel, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 2023−2026.
(21) Jessop, P. G.; Morris, R. H. *Inorg. Chem*. **1993**, *32*, 2236.
(22) Darensbourg, M. Y.; Ludvig, M. M. *Inorg. Chem.* **1986**, *25*, 2894−2898.
(23) Ovchinnikov, M. V.; Angelici, R. J. *J. Am. Chem. Soc.* **2000**, *122*, 6130.
(24) (a) Cao, Z.; Hall, M. B. *J. Am. Chem. Soc.* **2001**, *123*, 3734−3742. (b) Fan, H.-J.; Hall, M. B. *J. Am. Chem. Soc.* **2001**, *123*, 3828−3829.