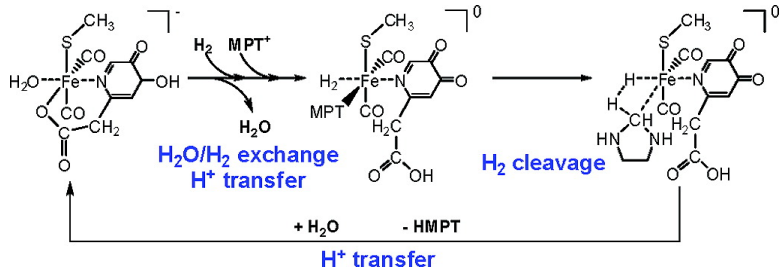
Communication

Trigger Mechanism for the Catalytic Hydrogen Activation by Monoiron (Iron#Sulfur Cluster-Free) Hydrogenase

Xinzheng Yang, and Michael B. Hall

J. Am. Chem. Soc., **2008**, 130 (43), 14036-14037 • DOI: 10.1021/ja804364p • Publication Date (Web): 01 October 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S COMMUNICATIONS

Published on Web 10/01/2008

Trigger Mechanism for the Catalytic Hydrogen Activation by Monoiron (Iron−Sulfur Cluster-Free) Hydrogenase

Xinzheng Yang and Michael B. Hall*

Department of Chemistry, Texas A&M University, College Station, Texas 77843-3255

Received June 9, 2008; E-mail: mbhall@tamu.edu

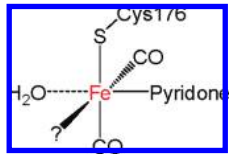
[NiFe]- and [FeFe]-hydrogenases have attracted increasing attention because their active sites use only base metals in the efficient reversible oxidation of molecular hydrogen. Knowledge of their structures and catalytic mechanisms are actively being applied to the development of efficient synthetic catalysts for hydrogen-fueled processes.[1]

A third type of phylogenetically unrelated hydrogenase, H_2-forming methylenetetrahydromethopterin dehydrogenase (Hmd, iron−sulfur cluster-free hydrogenase, [Fe]-hydrogenase), was discovered by Thauer and co-workers[2] in *Methanothermobacter marburgensis*. In contrast to the dimetal hydrogenases, Hmd catalyzes the reversible reduction of N^5, N^{10}-methenyl-tetrahydromethanopterin (methenyl-H_4MPT$^+$, or MPT$^+$) with H_2 to N^5, N^{10}-methylene-tetrahydromethanopterin (methylene-H_4MPT, or HMPT) and a proton (eq 1). A hydride is stereospecifically transferred from H_2 onto the *pro*-R side of MPT$^+$.[3] Hmd also catalyzes a direct exchange of the *pro*-R hydrogen of MPT with the protons of water. [4] It differs from the dimetal hydrogenases in that the purified enzyme *per se* does not catalyze the conversion of *para*-H_2 to *ortho*-H_2 or the isotopic H_2/D_2O exchange reaction. These reactions specifically require the presence of methenyl-H_4MPT$^+$.[4]

$$\text{Methenyl-H}_4\text{MPT}^+ + \text{H}_2 \overset{\text{Hmd}}{\rightleftharpoons} \text{Methylene-H}_4\text{MPT} + \text{H}^+ \qquad (1)$$

Although spectroscopic studies and the structure of the apoenzyme have been reported,[4] the key for the reaction modeling was the recent crystal structure of the active enzyme.[5] The active site iron atom has a pseudo-octahedral coordination with *cis*-$(CO)_2$, the S of Cys176 in the $(CO)_2$ plane, the pyridone ligand binding through N perpendicular to this plane, a H_2O *trans* to the pyridone, and an unknown ligand in the sixth site (Scheme 1).

Scheme 1. Model for Hmd Active Site[a]

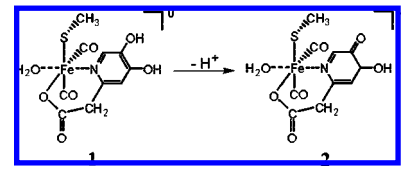


[a] The unknown ligand is "?".

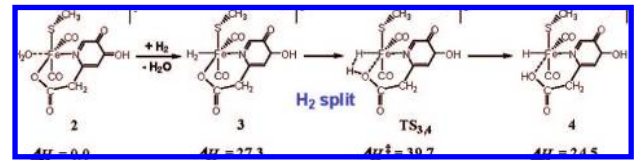
Because the splitting of H_2 between a strong base and a good hydride acceptor, such as a carbocation, is facile, it was widely accepted that this enzyme was "metal-free". Since the discovery that Fe is present in the active site, a suitable role for that Fe was sought. If Fe's role was simply to capture and hold the H_2 until MPT$^+$ arrives, the need for the pyridone appears lost (apart from a cyanide mimic).[5] Here, density functional theory calculations[6] (Computational Details are in Supporting Information (SI)) predict a trigger mechanism that invokes the Fe, the pyridone, and MPT$^+$ intimately in H_2 cleavage and hydride transfer.

Proposed resting states of the active-site models of Hmd are shown in Scheme 2 with different protonation states for the model pyridone

Scheme 2. Models of the Resting State Structure of Hmd



Scheme 3. Relative Enthalpies for Direct H_2 Splitting in **2** without MPT$^+$ (kcal/mol)



ligand. In the neutral structure **1**, the iron-free cofactor in the Hmd apoenzyme was simplified as the pyridone, $NC_5H_2(OH)_2CH_2COO^-$, bonding to Fe through the N, which is then deprotonated to form **2**. The position of an O atom attached to the ring closest to N was shifted one C position to prevent it from interacting strongly with the sulfur and *cis*-CO groups bonding with Fe, a problem that the enzyme may avoid through the protein. An O atom of the carboxyl in this ligand occupies the unknown ligand site of Fe and stabilizes the structure. However, the protein may provide an alternative ligand that performs the same function as the carboxylate in this study.

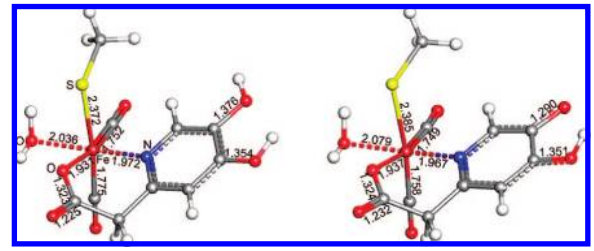


Figure 1. Optimized structures of **1** and **2**. Bond lengths are in angstrom.

The optimized structures of **1** and **2** are displayed in Figure 1. The decrease of total charge (**1** → **2**) increases the donor power of the pyridone and reduces the vibrational frequencies of the CO groups from 1980 and 2034 cm^{-1} in **1** to 1957 and 2014 cm^{-1} in **2**, the latter are about 20 cm^{-1} closer to the observed absorption peaks of Hmd's resting state at 1944 and 2011 cm^{-1},[4d] and the remaining frequency differences between **2** and Hmd are similar to those of model compounds (analysis is in SI).

H_2 activation with the model resting-state structure **2** in the absence of MPT$^+$ is shown in Scheme 3. The enthalpic barrier for H_2O/H_2 substitution (**2** → **3**) is 27.3 kcal/mol, and the transition state $\mathbf{TS_{4,5}}$ (1156*i* cm^{-1}) for the cleavage of H_2 is 12.4 kcal/mol above **3**. Therefore, the total enthalpic barrier for the cleavage of H_2 without

10.1021/ja804364p CCC: $40.75

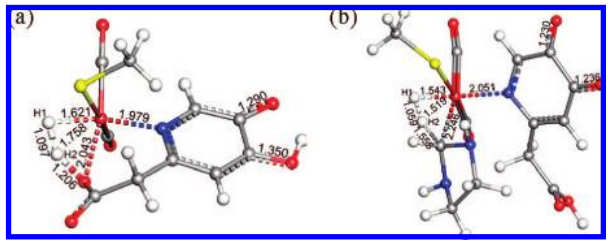
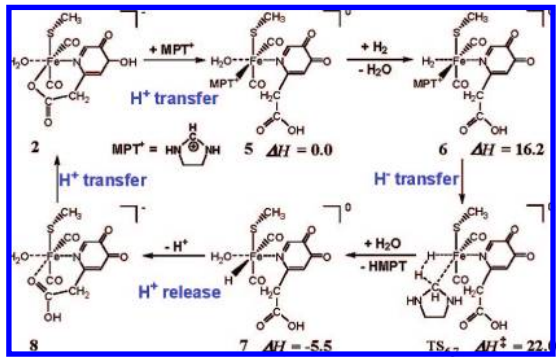


Figure 2. Optimized transition state structures for H_2 cleavage: (a) $\mathbf{TS_{3,4}}$ (1156*i* cm^{-1}), Mulliken charge on H1 and H2 are −0.163 and 0.276; (b) $\mathbf{TS_{6,7}}$ (951*i* cm^{-1}), Mulliken charge on H1 and H2 are −0.022 and 0.071.

Scheme 4. Mechanism with Relative Enthalpies for H_2 Cleavage Catalyzed by the Model Active Site with MPT^+ (kcal/mol)



MPT^+ is 39.7 kcal/mol, which is too high to be achieved at mild enzymatic conditions.

The mechanism for H_2 cleavage catalyzed by the model active site of Hmd in the presence MPT^+ is shown in Scheme 4. Here, simple deprotonation will be used to model the pyridone's access to a quinone-like structure to provide a temporarily reduced or partially reduced Fe. Beginning from the resting-state structure **2**, the appearance of MPT^+ forces a rotation of the carboxyl and stabilizes the quinone form, while the unsaturated carbon atom binds close to Fe in forming intermediate **5**.[7] When the simple MPT^+ model is used, the Fe−C distance is 2.049 Å and one can view the interaction as an Fe → C dative bond.[8] However, with a larger more realistic substrate, **5** shows a significantly longer Fe−C contact.

The Fe−MPT^+ interaction reduces the enthalpic barrier for H_2O/H_2 substitution to 16.2 kcal/mol (**5** → **6**). Furthermore, when MPT^+ is close to Fe as in **6**, H_2 is split easily through transition state $\mathbf{TS_{6,7}}$ with a barrier only 5.8 kcal/mol above **6**. Here, one H transfers to MPT^+ to form a neutral HMPT molecule, while the remaining H bonds to Fe. With loss of HMPT, H_2O enters and forces the remaining H toward the position vacated by HMPT to form intermediate **7**, which is 21.7 kcal/mol more stable than **6**. Then, the resting state **2** will be regenerated through proton transfer steps. Alternatively, H^+ could leave directly before H_2O recoordinates, and then the COOH group could recoordinate to the position vacated by HMPT. Since H_2 can substitute H_2O without the presence of MPT^+ (**2** → **3**), it is not essential that MPT^+ arrive first. Thus, an equally viable mechanism could involve **2** → **3** then **3** → **6**. The important point is that H_2 splitting and hence its *para*-H_2 to *ortho*-H_2 conversion or H/D exchange with D_2O will occur only in the presence of MPT^+.

The Mulliken atomic charges on the hydrogens for the H_2 cleavage transition state without MPT^+ ($\mathbf{TS_{3,4}}$, Figure 2a) are consistent with a typical heterolytic cleavage mechanism where a proton is transferred to the base, O in this case, and a hydride is transferred to the metal. The poor basicity of the coordinated O and the strong pyridone donor *trans* to the new H−Fe bond resist this transformation disfavoring the H_2 cleavage. The Mulliken atomic charges on hydrogens for the H_2 cleavage transition state in the presence of MPT^+ ($\mathbf{TS_{6,7}}$, Figure 2b) are quite different as both are nearly neutral. In this delocalized transition state, the strong donor power of the pyridone is essential as the electron density from the pyridone is being transferred through Fe to both the MPT^+ and the new H−Fe bond; formally this H migrates to Fe as a proton because MPT^+ takes the hydride. The large role played by electron donation from the pyridone is visible in the shortened C═O (quinone-like) bonds in $\mathbf{TS_{6,7}}$ compared to $\mathbf{TS_{3,4}}$.

In summary, a fully optimized model for the resting state of the active site of the monoiron (iron−sulfur cluster-free) hydrogenase is proposed. Based on this model, a mechanism for the H_2 activation with MPT^+ catalyzed by Hmd was predicted. The exchange of H_2/H^+ catalyzed by Hmd is strictly dependent on the presence of MPT^+. The ability of the pyridone to change its donor ability is a key to the "trigger" mechanism proposed here. Its strong donor strength prevents the usual heterolytic cleavage from occurring, and only in the presence of MPT^+ does the donor strength of the pyridone efficiently assist the transfer of H and electron density (effectively H^-) to the MPT^+. MPT^+ and H_2 can arrive at the active site in any order, but both must be present for H_2 activation. The enzyme plays a key role in mediating the donor strength of the pyridone, here modeled simply by deprotonation. The ortho position of the two O atoms assists in the increase of the pyridone donor strength. We are currently exploring alternative O positions corresponding to those in the crystal structures. The understanding provided by the proposed catalytic mechanism for hydrogen activation will be important for the further design of novel hydrogenation catalysts, and perhaps for low cost, high efficiency hydrogen production and for release from hydrogen storage materials.[9]

Acknowledgment. This work was supported by grants from the NSF (CHE-0518074, CHE-0541587, and DMS-0216275) and The Welch Fundation (A0648).

Supporting Information Available: Complete ref 6, computational details, and atomic coordinates and absolute energies of optimized stationary points and transition states. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Thauer, R. K.; Klein, A. R.; Hartmann, G. C. *Chem. Rev.* **1996**, *96*, 3031. (b) Kubas, G. J. *Chem. Rev.* **2007**, *107*, 4152. (c) Vignais, P. M.; Billoud, B. *Chem. Rev.* **2007**, *107*, 4206. (d) Fontecilla-Camps, J. C.; Volbeda, A.; Cavazza, C.; Nicolet, Y. *Chem. Rev.* **2007**, *107*, 4273. (e) De Lacey, A. L.; Fernández; V. M.; Rousset, M.; Cammack, R. *Chem. Rev.* **2007**, *107*, 4304. (f) Vincent, K. A.; Parkin, A.; Armstrong, F. A. *Chem. Rev.* **2007**, *107*, 4366. (g) Siegbahn, P. E. M.; Tye, J. W.; Hall, M. B. *Chem. Rev.* **2007**, *107*, 4414. (h) Thomas, C. M.; Rudiger, O.; Liu, T.; Carson, C. E.; Hall, M. B.; Darensbourg, M. Y. *Organometallics* **2007**, *26*, 3976.
(2) Zirngibl, C.; Hedderich, R.; Thauer, R. K. *FEBS Lett.* **1990**, *261*, 112.
(3) Schleucher, J.; Griesinger, C.; Schröder, B.; Thauer, R. K. *Biochemistry* **1994**, *33*, 3986.
(4) (a) Hartmann, G. C.; Santamaria, E.; Fernandez, V. M.; Thauer, R. K. *J. Biol. Inorg. Chem.* **1996**, *1*, 446. (b) Shima, S.; Thauer, R. K. *Chem. Rec.* **2007**, *7*, 37. (c) Pilak, O.; Mamat, B.; Vogt, S.; Hagemeier, C. H.; Thauer, R. K.; Shima, S.; Vonrhein, C.; Warkentin, E.; Ermler, U. *J. Mol. Biol.* **2006**, *358*, 798. (d) Lyon, E. J.; Shima, S.; Boecher, R.; Thauer, R. K.; Grevels, F.-W.; Bill, E.; Roseboom, W.; Albracht, S. P. J. *J. Am. Chem. Soc.* **2004**, *126*, 14239. (e) Guo, Y.; Wang, H.; Xiao, Y.; Vogt, S.; Thauer, R. K.; Shima, S.; Volkers, P. I.; Rauchfuss, T. B.; Pelmenschikov, V.; Case, D. A.; Alp, E. E.; Sturhahn, W.; Yoda, Y.; Cramer, S. P. *Inorg. Chem.* **2008**, *47*, 3969.
(5) Shima, S.; Pilak, O.; Vogt, S.; Schick, M.; Stagni, M. S.; Meyer-Klaucke, W.; Warkentin, E.; Thauer, R. K.; Ermler, U. *Science* **2008**, *321*, 572.
(6) Frisch, M. J. et al. *Gaussian 03*, revision C.02; Gaussian, Inc.: Pittsburgh, PA, 2003.
(7) Because it is known that the protein binds MPT^+, we have chosen the reference enthalpy as **5**, which avoids the artificial charge stabilization of the gas-phase reaction. We are currently studying larger MPT^+ models and the effect of protein on the energy of MPT^+ binding. Based on preliminary solvent energy calculations of larger MPT^+ models, we estimate that the enthalpy for **2** + MPT^+ → **5** is ±5 kcal/mol (see SI).
(8) Parkin, G. *Organometallics* **2006**, *25*, 4744.
(9) Yang, X.; Hall, M. B. *J. Am. Chem. Soc.* **2008**, *130*, 1798.

JA804364P