Hydrogenases

DOI: 10.1002/anie.200900494

Influence of the $[2Fe]_H$ Subcluster Environment on the Properties of Key Intermediates in the Catalytic Cycle of [FeFe] Hydrogenases: Hints for the Rational Design of Synthetic Catalysts**

Maurizio Bruschi, Claudio Greco, Markus Kaukonen, Piercarlo Fantucci, Ulf Ryde,* and Luca De Gioia**

A peculiar Fe_6S_6 cluster, referred to as the H-cluster, is found in the active site of [FeFe] hydrogenases. The H-cluster can be described as a classical Fe_4S_4 cluster that is bridged, through the sulfur atom of a cysteine residue, to a biologically unusual binuclear Fe cluster, usually referred to as the $[2Fe]_H$ subcluster.[1] In the subcluster, the two iron ions are bridged by a S-CH_2-X-CH_2-S ligand. Mechanistic considerations[2] and computation of energy barriers[3] support the presence of di(thiomethyl)amine (dtma, X = NH) as the chelating ligand. However, it has been also proposed that X might correspond to CH_2 (propane-1,3-dithiolate, pdt) or O (di(thiomethyl) ether, dtme).[1,4]

It has been noted that, in principle, intermediates with both terminal and bridging hydride ligands in $[2Fe]_H$ might be formed in the catalytic cycle of [FeFe] hydrogenases leading to H_2 formation.[5] Indeed, investigations of models of the $[2Fe]_H$ subcluster revealed that the thermodynamically most stable forms generally correspond to μ-hydride species.[6] However, experimental results[7] and DFT calculations[3] have shown that only terminal-hydride species are sufficiently reactive in H_2 production, corroborating the hypothesis that only terminal-hydride species are transiently formed in the [FeFe]-hydrogenase catalytic cycle.[3,5] In this scenario, a question particularly relevant not only to better understanding of the chemistry of [FeFe] hydrogenases, but also for the design of biomimetic synthetic catalysts, is why unreactive μ-hydride species are not formed in the enzyme active site. Since dinuclear synthetic models and the $[2Fe]_H$ subcluster differ mainly in their environment (bulk solvent versus Fe_4S_4 cluster and neighboring amino acids), a reasonable hypothesis implies that terminal-hydride species in the enzyme are selectively stabilized by the environment of the $[2Fe]_H$ cluster. Another reasonable hypothesis implies that in the enzyme the reaction of terminal-hydride species with protons and electrons is significantly faster than isomerization to μ-hydride forms; in this scenario the environment of the $[2Fe]_H$ cluster might also play a crucial role.

With the aim of better defining the influence of the $[2Fe]_H$ subcluster environment on the properties of key intermediate species formed in the catalytic cycle of [FeFe] hydrogenases, we used density functional theory (DFT) and combined quantum and molecular mechanics calculations (QM/MM) to investigate key hydride species formed in the catalytic cycle, taking explicitly into account the presence of the amino acid environment and the Fe_4S_4 cluster, which is the most proximal group interacting with the $[2Fe]_H$ subcluster and has been shown to affect the stereoelectronic properties of the binuclear cluster (Figure 1).[8–10]

The catalytically relevant species that have been taken into account in this study are summarized in Scheme 1. For each complex, four different models have been considered. In the first model, the Fe_4S_4 cluster was simply modeled by

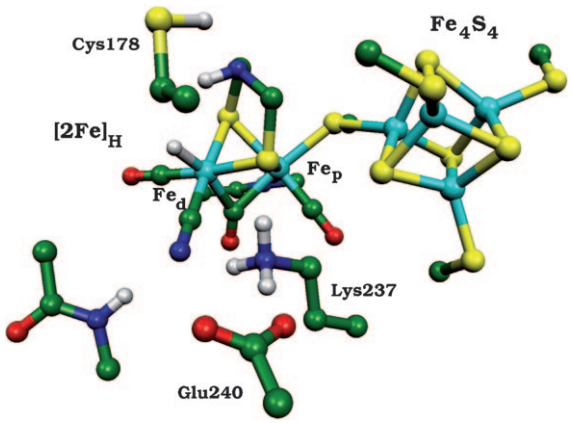
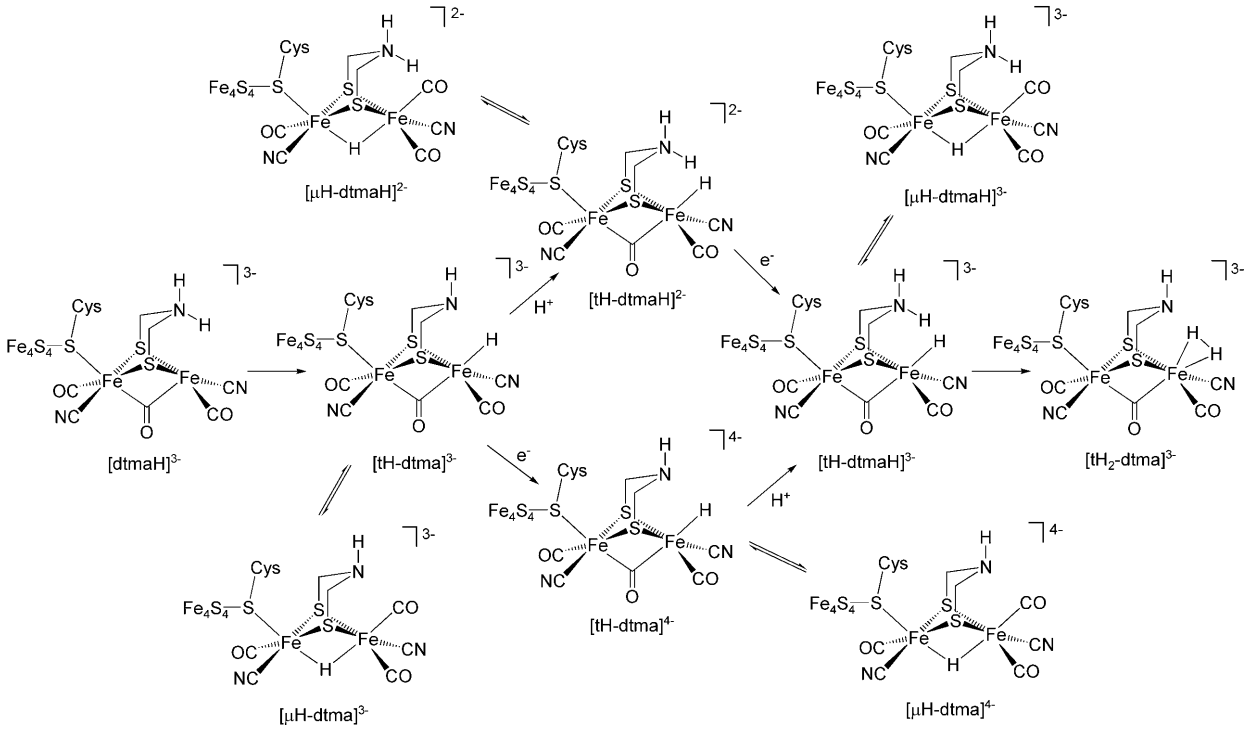


Figure 1. Molecular geometry of the H-cluster with the surrounding amino acid residues included in *Mod-3* (numbered according to the 1HFE pdb file). The proximal and distal (relative to the Fe_4S_4 cluster) iron atoms of the $[2Fe]_H$ subcluster are referred to as Fe_p and Fe_d, respectively. Fe teal, S yellow, C green, N blue, O red, H gray.

[*] Dr. M. Bruschi
Department of Environmental Science
University of Milano-Bicocca
Piazza della Scienza 1, 20126 Milan (Italy)
Fax: (+39) 02-6448-2890
E-mail: maurizio.bruschi@unimib.it
Dr. M. Kaukonen, Prof. U. Ryde
Department of Theoretical Chemistry, Lund University
P.O. Box 124, 221 00 Lund (Sweden)
Fax: (+46) 46-222-4543
E-mail: ulf.ryde@theokem.lu.se
Dr. C. Greco, Prof. P. Fantucci, Prof. L. De Gioia
Department of Biotechnology and Biosciences
University of Milano-Bicocca
Piazza della Scienza 2, 20126 Milan (Italy)
Fax: (+39) 02-6448-3478
E-mail: luca.degioia@unimib.it

[**] We are grateful to the Lunarc center for scientific and technical computing for research at Lund University. M.K. is grateful for financial support by the MIDE program of Helsinki University of Technology.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200900494.



Scheme 1. Plausible intermediate species which might be formed in the reaction path leading to H_2 formation.

protonation of the sulfur atom of the CH_3S group (hereafter referred to *Mod-1*). The second model (*Mod-2*) includes the entire H-cluster, in which the four sulfur atoms of the cysteine residues coordinated to the Fe_4S_4 core are constrained to their X-ray positions.[1b] The third model (*Mod-3*) corresponds to the H-cluster plus the constrained side chains of Lys237, Glu241, Cys178, and the backbone atoms of Pro108, Ala109, Pro203, and Ile204, which surround the $[2Fe]_H$ cluster (residue numbers from the 1HFE PDB file).[1b] Finally, the fourth model (*Mod-4*) corresponds to the entire protein in the framework of a QM/MM approach[11] (see the Supporting Information for a more detailed definition of the models). In all models, X of the S-CH_2-X-CH_2-S chelating ligand has been assumed to be an amine group, which may either be protonated (NH_2, complexes labeled with "dtmaH") or deprotonated (NH, complexes labeled with "dtma").

It was suggested that the first terminal-hydride species in the H_2 formation pathway is obtained upon proton transfer from protonated dtma to the Fe_d atom of the Fe^IFe^I $[2Fe]_H$ cluster.[10] Therefore, the first catalytically relevant species taken into account is $[Fe_4S_4(SCH_3)_4FeFe(CO)_3(CN)_2(dtmaH)]^{-3}$ (Scheme 1, $[dtmaH]^{3-}$). Analysis of spin densities and partial charges of $[dtmaH]^{3-}$ (Tables S4 and S5 in the Supporting Information) show that, independent of the computational model, the $[2Fe]_H$ and Fe_4S_4 clusters are in the Fe^IFe^I and +2 redox states, respectively, in agreement with Mössbauer spectroscopy data.[12,13] The complex $[dtmaH]^{3-}$ evolves according to a strongly exothermic reaction step (Table 1) in which the proton is transferred from dtmaH to the Fe_d atom of the $[2Fe]_H$ subcluster to form the metal hydride complex $[Fe_4S_4(SCH_3)_4FeFeH(CO)_3(CN)_2(dtma)]^{-3}$ ($[H\text{-}dtma]^{3-}$; Scheme 1).[14] Analysis of spin and charge densities reveals that on going from $[dtmaH]^{3-}$ to $[H\text{-}dtma]^{3-}$, the Fe_4S_4 core remains in the +2 oxidation state, while the $[2Fe]_H$ cluster is formally oxidized from Fe^IFe^I to $Fe^{II}Fe^{II}$. Remarkably, the μ-hydride isomer $[\mu H\text{-}dtma]^{3-}$ is predicted to be significantly more stable than the terminal-hydride species $[tH\text{-}dtma]^{3-}$, even when the surrounding amino acids are explicitly considered (*Mod-3* and *Mod-4*, Table 1), thus indicating that the environment does not selectively stabilize the terminal-hydride isomer $[Fe_4S_4(SCH_3)_4FeFeH(CO)_3(CN)_2(dtma)]^{-3}$.

The next step in the catalytic cycle can be either protonation of dtma leading to $[H\text{-}dtmaH]^{2-}$ (in which the Fe_4S_4 cluster remains in the +2 redox state; Tables S4 and S5 in the Supporting Information) or one-electron reduction of

Table 1: Energy difference ΔE (kcal mol^{-1}) between catalytically relevant isomers.[a]

	Mod-1	*Mod-2*	*Mod-3*	*Mod-4*
$[dtmaH]^{3-} \rightarrow [tH\text{-}dtma]^{3-}$	−9.7	−14.5	−11.9	−14.3
$[tH\text{-}dtma]^{3-} \rightarrow [\mu H\text{-}dtma]^{3-}$	−11.4	−8.9	−9.3	−9.4
$[tH\text{-}dtmaH]^{2-} \rightarrow [\mu H\text{-}dtmaH]^{2-}$	−8.7	−5.9	−5.3	−2.0
$[tH\text{-}dtma]^{4-} \rightarrow [\mu H\text{-}dtma]^{4-}$	−3.9	−5.8	−4.7	−0.6
$[tH\text{-}dtmaH]^{3-} \rightarrow [\mu H\text{-}dtmaH]^{3-}$	−1.0	−0.7	−0.5	+0.5

[a] For *Mod-1*, *Mod-2*, and *Mod-3*, geometry optimizations and energy calculations were carried out using the BP86/def-TZVP computational scheme. For *Mod-4*, geometry optimizations and energy calculations were carried out using the BP86/SVP computational scheme for the QM part and the Amber1999 force field for the MM part (see the Supporting Information for a detailed discussion of the computational methods). Geometry optimization (for *Mod-1* and *Mod-2*) and single-point QM/MM energy calculations (for *Mod-4*) were also performed at the B3LYP/def-TZVP level. The results are reported in the Supporting Information.

$[H\text{-}dtma]^{3-}$ to give $[H\text{-}dtma]^{4-}$ (Scheme 1). When considering the protonated-dtma forms $[tH\text{-}dtmaH]^{2-}$ and $[\mu H\text{-}dtmaH]^{2-}$ (Scheme 1), the μ-hydride isomer is still lower in energy than the terminal-hydride one, even if the energy difference is smaller than that calculated for the $[H\text{-}dtma]^{3-}$ isomers (Table 1). Moreover, the environment of the $[2Fe]_H$ subcluster (Fe_4S_4 and protein) affects the relative stability of the two isomers. In fact, the energy difference decreases from about 9 to 2 kcal mol^{-1} going from *Mod-1* to *Mod-4* (Table 1).

When considering the reduced intermediate $[H\text{-}dtma]^{4-}$, the μ-hydride isomer is still thermodynamically more stable than the terminal-hydride form ($[\mu H\text{-}dtma]^{4-}$ and $[tH\text{-}dtma]^{4-}$; Scheme 1 and Table 1), even though the energy gap is further decreased with respect to the values calculated for $[H\text{-}dtma]^{3-}$ and $[H\text{-}dtmaH]^{2-}$ isomers. Notably, when the entire H-cluster is considered (*Mod-2*, *Mod-3*, *Mod-4*), in both $[\mu H\text{-}dtma]^{4-}$ and $[tH\text{-}dtma]^{4-}$ the unpaired electron is mainly localized on the Fe_4S_4 cluster (Tables S4 and S5 in the Supporting Information), which therefore can be formally assigned to the +1 redox state, analogous to models of the H-cluster in which dtma is replaced by pdt.[10] Moreover, for the models including the entire H-cluster, the $[2Fe]_H$ cluster, which remains in the $Fe^{II}Fe^{II}$ redox state, is more similar to the dinuclear model (*Mod-1*) of $[H\text{-}dtma]^{3-}$ rather than $[H\text{-}dtma]^{4-}$, which is reduced to the $Fe^{I}Fe^{II}$ redox state. Interestingly, the effect of the environment on the relative stability of the two isomers, if compared to that of the dinuclear model (*Mod-1*) of $[H\text{-}dtma]^{3-}$, is significant, as the energy difference decreases from about 11 to about 1 kcal mol^{-1} going from *Mod-1* to *Mod-4* (Table 1).

The next step in the catalytic cycle should either correspond to protonation of dtma in $[H\text{-}dtma]^{4-}$ or one-electron reduction of $[H\text{-}dtmaH]^{2-}$. In both cases the intermediate $[H\text{-}dtmaH]^{3-}$ is formed (Scheme 1). As shown in Table 1, $[\mu H\text{-}dtmaH]^{3-}$ and $[tH\text{-}dtmaH]^{3-}$ are almost isoenergetic, irrespective of the adopted model. Therefore, also in $[H\text{-}dtmaH]^{3-}$ long-range effects owing to the protein do not significantly affect the relative stability of the two isomers.

Protonation of dtma in $[H\text{-}dtma]^{4-}$, leading to $[H\text{-}dtmaH]^{3-}$, is accompanied by electron transfer from the Fe_4S_4 cluster to the $[2Fe]_H$ cluster. In fact, in $[H\text{-}dtmaH]^{3-}$ the unpaired electron is localized on the $[2Fe]_H$ cluster, which is reduced to the formal $Fe^{II}Fe^{I}$ redox state, while the Fe_4S_4 cluster is oxidized from the +1 to the +2 redox state (see Tables S4 and S5 in the Supporting Information). Therefore, the protonation/deprotonation of dtma promotes electron transfer between the two subunits of the H-cluster, well illustrating how proton and electron transfers can be strongly coupled in the H-cluster.

One-electron reduction of $[H\text{-}dtmaH]^{2-}$ to $[H\text{-}dtmaH]^{3-}$ leads to an increased negative charge on the hydride ion, which therefore is expected to interact more strongly with the NH_2^+ group of dtmaH, resulting in facile H_2 formation.[15] In fact, in $[tH\text{-}dtmaH]^{3-}$, the distance between the ammonium hydrogen atom of dtma and the hydride bound to Fe_d is extremely short (1.34 Å).

In summary, the DFT and QM/MM analysis of metal hydride species relevant to the [FeFe] hydrogenase catalytic cycle clearly shows that the protein matrix and the proximal Fe_4S_4 cluster either do not play any role, as in $[H\text{-}dtma]^{3-}$, or play a minor but not crucial role, as in $[H\text{-}dtmaH]^{2-}$ and $[H\text{-}dtma]^{4-}$, in determining the relative thermodynamic stability of $[2Fe]_H$ hydride intermediate species. These results, also corroborated by B3LYP data (see the Supporting Information), lead to the conclusion that terminal-hydride species in the enzyme active site are also thermodynamically less stable than the corresponding μ-hydride forms.

In light of these observations, formation and reactivity of terminal-hydride species in the enzyme active site should be under kinetic control, that is, reaction with electrons and protons leading to H_2 formation must be considerably faster than terminal- to μ-hydride isomerization. In this scenario, as also suggested by others,[16] a crucial role in the kinetic trapping of terminal-hydride intermediates formed in the enzymatic catalytic cycle could be played by the residue Lys237, which is strictly conserved in [FeFe] hydrogenases and forms a salt-bridge network involving the CN group coordinated to Fe_d (see the Supporting Information). In fact, the electrostatic interaction between the positively charged side chain of Lys237 and the CN^- ligand might restrain rotation of the $Fe_d(CO)_2(CN)$ group, possibly kinetically hindering isomerization from terminal- to μ-hydride forms in the protein. The "freezing" effect of Lys237 could be difficult to reproduce in bioinspired synthetic catalysts, mainly because CN^- ligands are generally avoided, as they compete with iron for protonation.[17] However, the use of tailored bulky or constrained ligands could be an alternative and functionally equivalent strategy to kinetically hinder the conversion between terminal- and μ-hydride species.[18]

Another hint for the design of synthetic catalysts that can be taken from the analysis of the $[2Fe]_H$ subcluster enzyme active site is related to the energy difference between unreactive μ-hydride species and reactive terminal-hydride species as a function of the subcluster redox state and protonation state of the chelating dtma ligand. Analysis of simple dinuclear models of the H-cluster (Table 1, *Mod-1*) reveals that the energy difference between μ- and terminal-hydride isomers decreases upon protonation of dtma and concomitant reduction of the binuclear cluster (also confirmed using the B3LYP functional; see the Supporting Information). The implications of this remarkable observation for the reactivity of synthetic dinuclear clusters inspired by the [FeFe] hydrogenase active site are intriguing and worth exploring.

Received: January 26, 2009
Published online: April 6, 2009

Keywords: bioinorganic chemistry · density functional calculations · enzyme catalysis · hydrogen · quantum chemistry

[1] a) J. W. Peters, W. N. Lanzilotta, B. J. Lemon, L. C. Seefeldt, *Science* **1998**, *282*, 1853 – 1858; b) Y. Nicolet, C. Piras, P. Legrand, E. C. Hatchikian, J. C. Fontecilla-Camps, *Structure* **1999**, *7*, 13 – 23.

[2] a) Y. Nicolet, B. J. Lemon, J. C. Fontecilla-Camps, J. W. Peters, *Trends Biochem. Sci.* **2000**, *25*, 138 – 143; b) Y. Nicolet, A. L. de Lacey, X. Vernède, V. M. Fernandez, E. C. Hatchikian, J. C. Fontecilla-Camps, *J. Am. Chem. Soc.* **2001**, *123*, 1596 – 1601.

[3] a) H. J. Fan, M. B. Hall, *J. Am. Chem. Soc.* **2001**, *123*, 3828 – 3829; b) G. Zampella, C. Greco, P. Fantucci, L. De Gioia, *Inorg. Chem.* **2006**, *45*, 4109 – 4118.

[4] A. S. Pandey, T. V. Harris, L. J. Giles, J. W. Peters, R. K. Szilagyi, *J. Am. Chem. Soc.* **2008**, *130*, 4533 – 4540.

[5] a) M. Bruschi, G. Zampella, P. Fantucci, L. De Gioia, *Coord. Chem. Rev.* **2005**, *249*, 1620 – 1640; b) P. E. M. Siegbahn, J. W. Tye, M. B. Hall, *Chem. Rev.* **2007**, *107*, 4414 – 4435; c) M. H. Cheah, S. Tard, S. J. Borg, X. Liu, S. K. Ibrahim, C. J. Pickett, S. P. Best, *J. Am. Chem. Soc.* **2007**, *129*, 11085 – 11092.

[6] a) M. Bruschi, P. Fantucci, L. De Gioia, *Inorg. Chem.* **2003**, *42*, 4773 – 4781; b) F. Wang, M. Wang, X. Liu, K. Jin, W. Dong, L. Sun, *J. Chem. Soc. Dalton Trans.* **2007**, 3812 – 3819; c) X. Zhao, I. P. Georgakaki, M. L. Miller, R. Mejia-Rodriguez, C.-Y. Chiang, M. Y. Darensbourg, *Inorg. Chem.* **2002**, *41*, 3917 – 3928; d) X. Zhao, C.-Y. Chiang, M. L. Miller, M. V. Rampersad, M. Y. Darensbourg, *J. Am. Chem. Soc.* **2003**, *125*, 518 – 524.

[7] a) J. I. Van der Vlugt, T. B. Rauchfuss, C. M. Whaley, S. R. Wilson, *J. Am. Chem. Soc.* **2005**, *127*, 16012 – 16013; b) S. Ezzaher, J.-F. Capon, F. Gloaguen, F. Y. Petillon, P. Schollhammer, J. Talarmin, R. Pichon, N. Kervarec, *Inorg. Chem.* **2007**, *46*, 3426 – 3428.

[8] A. T. Flieder, T. C. Brunold, *Inorg. Chem.* **2005**, *44*, 9322 – 9334.

[9] D. E. Schwab, C. Tard, J. W. Peters, C. J. Pickett, R. K. Szilagyi, *Chem. Commun.* **2006**, 3696 – 3698.

[10] M. Bruschi, C. Greco, P. Fantucci, L. De Gioia, *Inorg. Chem.* **2008**, *47*, 6056 – 6071.

[11] a) U. Ryde, *J. Comput.-Aided Mol. Des.* **1996**, *10*, 153 – 164; b) U. Ryde, M. H. M. Olsson, *Int. J. Quantum Chem.* **2001**, *81*, 335 – 374.

[12] a) C. V. Popescu, E. Münck, *J. Am. Chem. Soc.* **1999**, *121*, 7877 – 7884; b) A. S. Pereira, P. Tavares, I. Moura, J. J. G. Moura, B. H. Huynh, *J. Am. Chem. Soc.* **2001**, *123*, 2771 – 2782.

[13] In the dinuclear complex (*Mod-1*), only an isomer with a semibridged CO ligand is stable with both the BP86 and B3LYP functionals. For all H-cluster models (*Mod-2*, *Mod-3*, and *Mod-4*), the two functionals give different results. In the case of BP86, two isomers featuring a μ-CO ligand or all-terminal CO ligands (rotated $[dtmaH]^{3-}$ or eclipsed $[dtmaH']^{3-}$ conformations) have been identified; the latter are slightly more stable than the former (1–2 kcal mol^{-1}). In the case of B3LYP, only one isomer featuring a semibridged CO ligand was identified.

[14] The possibility that $[dtmaH]^{3-}$ could undergo one-electron reduction before proton transfer from dtmaH to Fe_d has not been taken into account because the reduction of the H_{red} form has been shown to proceed at very low potentials and leads to degradation of the H-cluster (see S. P. J. Albracht, W. Roseboom, C. E. Hatchikian, *J. Biol. Inorg. Chem.* **2006**, *11*, 88 – 101).

[15] Proton transfer to give the terminal H_2 adduct ($[tH_2\text{-}dtma]^{-3}$), calculated for *Mod-1*, *Mod-2*, and *Mod-3*, is exothermic by about 5 kcal mol^{-1}.

[16] B. E. Thomas, T. B. Rauchfuss, *Inorg. Chem.* **2008**, *47*, 2261 – 2263; C. M. Thomas, T. Liu, M. B. Hall, M. Y. Darensbourg, *Inorg. Chem.* **2008**, *47*, 7009 – 7024.

[17] G. Eilers, L. Schwartz, M. Stein, G. Zampella, L. De Gioia, S. Ott, R. Lomoth, *Chem. Eur. J.* **2007**, *13*, 7075 – 7082; J.-F. Capon, F. Gloaguen, F. Y. Petillon, P. Schollhammer, J. Talarmin, *Eur. J. Inorg. Chem.* **2008**, 4671 – 4681.

[18] M. L. Singleton, N. Bhuvanesh, J. H. Reibenspies, M. Y. Darensbourg, *Angew. Chem.* **2008**, *120*, 9634 – 9637; *Angew. Chem. Int. Ed.* **2008**, *47*, 9492 – 9495; T. B. Liu, M. Y. Darensbourg, *J. Am. Chem. Soc.* **2007**, *129*, 7008 – 7009; A. K. Justice, M. J. Nilges, T. B. Rauchfuss, S. R. Wilson, L. De Gioia, G. Zampella, *J. Am. Chem. Soc.* **2008**, *130*, 5293 – 5301; A. K. Justice, T. B. Rauchfuss, S. R. Wilson, *Angew. Chem.* **2007**, *119*, 6264 – 6266; *Angew. Chem. Int. Ed.* **2007**, *46*, 6152 – 6154.

