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

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Role of the $[2Fe]_H$ subcluster environment on the properties of key intermediate species formed in the catalytic cycle of [FeFe] hydrogenases. Hints for the rational design of efficient synthetic catalysts.

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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, via 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 $S\text{-}CH_2\text{-}X\text{-}CH_2\text{-}S$ ligand. Mechanistic considerations[2] and computation of energy barriers[3] support the presence of dtma (X = NH) as the chelating ligand. However, it has been also proposed that the X group might correspond to CH_2 (pdt) or O (dtme).[1,4]

It was previously noted that, in principle, both terminal- and μ-hydride $[2Fe]_H$ intermediates 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 understand the chemistry of [FeFe] hydrogenases, but also for the design of biomimetic synthetic catalysts, is related to elucidate why unreactive μ-H species are not formed in the enzyme active site. Since dinuclear synthetic models and the $[2Fe]_H$ subcluster differ mainly for their environment (bulk solvent versus Fe_4S_4 cluster + 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 reactivity of terminal-hydride species with protons and electrons is significantly faster than isomerization to μ-hydride forms, and also in this scenario the environment of the $[2Fe]_H$ cluster might play a crucial role.

With the aim of better defining the role of the $[2Fe]_H$ subcluster environment on the properties of key intermediate species formed in the catalytic cycle of [FeFe] hydrogenases, we have 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 also explicitly into account the presence of the amino acid environment, as well as of 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 (see Figure 1). [8-10]

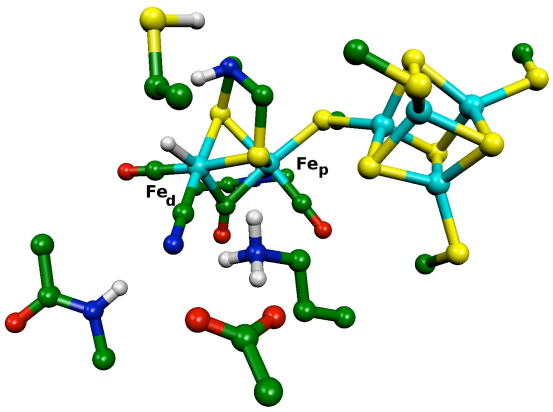

Figure 1. Molecular geometry of the H-cluster with the surrounding residues included in Mod-3. The proximal and distal (relative to the Fe_4S_4 cluster) iron atoms of the $[2Fe]_H$ subcluster are referred to as Fe_p and Fe_d, respectively.

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 modelled by protonation of the sulphur atom of the CH_3S group (hereafter indicated as 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 position.[1b] The third model (Mod-3) corresponds to the H-cluster plus the constrained side chains of Lys-237, Glu-241, Cys-178, and the backbone atoms of Pro-108, Ala-109, Pro-203, and Ile-204, 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 Supporting Information for a more detailed definition of the models).

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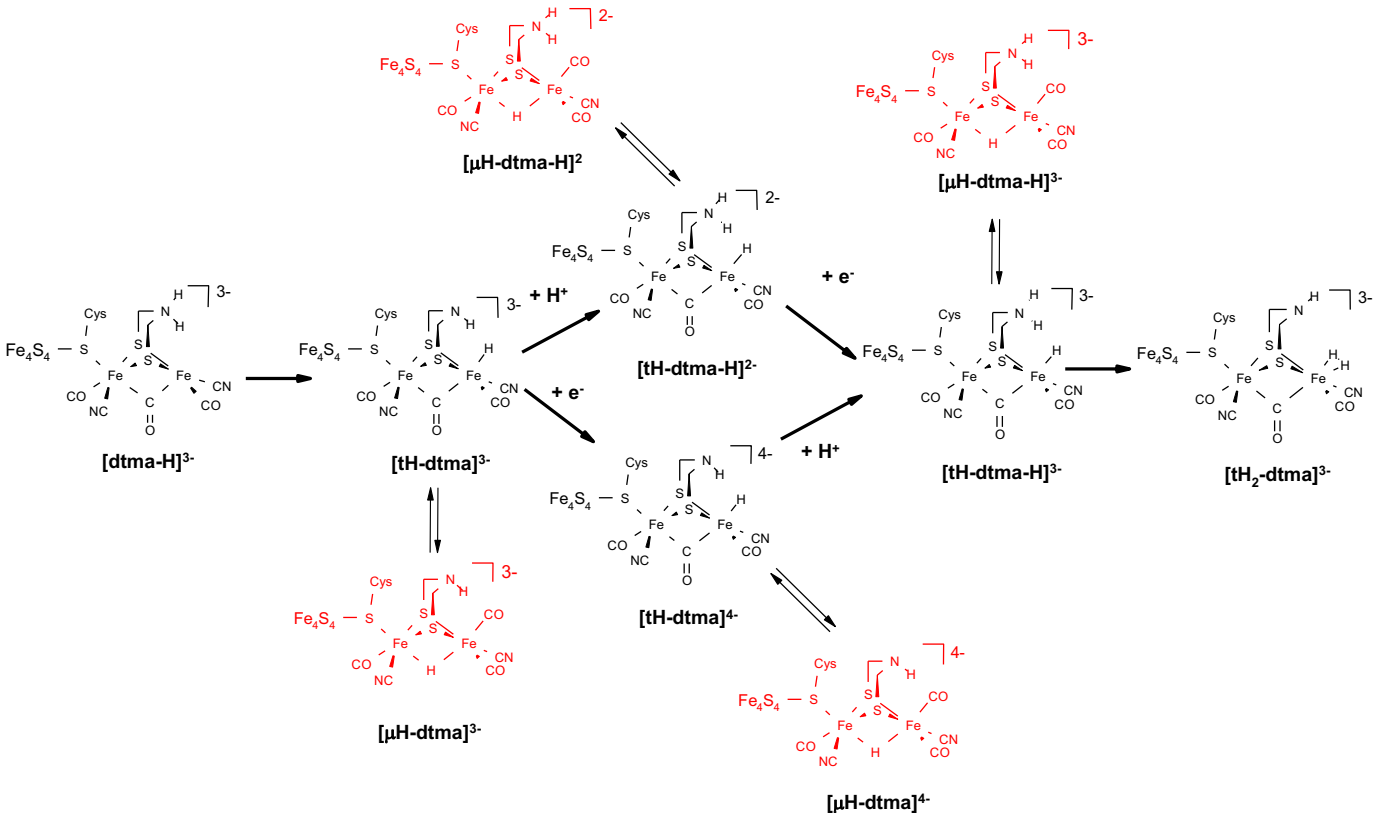

Scheme 1. Plausible intermediate species formed in the reaction path leading to H_2 formation. μ-hydride isomers are coloured in red to better distinguish them from terminal-hydride species.

In all models, the X atom of the S-CH_2-X-CH_2-S chelating ligand has been assumed to be a nitrogen atom, which may either be protonated (complexes labelled with the suffix "dtmaH") or deprotonated (complexes labelled with the suffix "dtma").

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

	Mod-1	*Mod-2*	*Mod-3*	*Mod-4*
[dtmaH]$^{3-}$ → [tH-dtma]$^{3-}$	-9.7	-14.5	-11.9	-14.3
[tH-dtma]$^{3-}$ → [μH-dtma]$^{3-}$	-11.4	-8.9	-9.3	-9.4
[tH-dtmaH]$^{2-}$→ [μH-dtmaH]$^{2-}$	-8.7	-5.9	-5.3	-2.0
[tH-dtma]$^{4-}$ → [μH-dtma]$^{4-}$	-3.9	-5.8	-4.7	-0.6
[tH-dtmaH]$^{3-}$→ [μH-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 Supporting Information for a detailed discussion of the computational methods). Geometry optimizations and energy calculations have also been performed for *Mod-1*, *Mod-2*, and *Mod-4* at the B3LYP/def-TZVP level of theory. The results are reported in the Supporting Information.

It was previously suggested that the first terminal-hydride species formed in the H_2 formation pathway is obtained upon proton transfer from protonated dtma to the Fe_d atom of the Fe(I)Fe(I) $[2Fe]_H$ cluster.[10] Therefore, the first catalytically relevant species that was taken into account is the $[Fe_4S_4(SCH_3)_4$-$FeFe(CO)_3(CN)_2(dtmaH)]^{-3}$ complex (Scheme 1, **[dtmaH]$^{3-}$**). Analysis of spin densities and partial charges of **[dtmaH]$^{3-}$** (see Table S3 and S4, respectively) show that, independently from the computational model, the $[2Fe]_H$ and the Fe_4S_4 clusters are in the Fe(I)Fe(I) and +2 redox state, respectively, in agreement with Mössbauer data.[12,13] **[dtmaH]$^{3-}$** evolves according to a strongly exothermic reaction step (see Table 1), in which the proton is transferred from dtmaH to the Fe_d atom of the $[2Fe]_H$ subcluster, forming the metal-hydride complex $[Fe_4S_4(SCH_3)_4Fe$-$FeH(CO)_3(CN)_2(dtma)]^{-3}$ (**[H-dtma]$^{3-}$**; Scheme 1).[14] Analysis of spin and charge densities (Table S3 and S4) reveals that going from **[dtmaH]$^{3-}$** to **[H-dtma]$^{3-}$**, the Fe_4S_4 core remains in the +2 oxidation state, while the $[2Fe]_H$ cluster is formally oxidized from Fe(I)Fe(I) to Fe(II)Fe(II). Remarkably, the μ-hydride isomer **[μH-dtma]$^{3-}$** is predicted to be significantly more stable than the terminal-hydride **[tH-dtma]$^{3-}$**, even when the surrounding amino acids are explicitly considered (Mod-3 and Mod-4, Table 1), indicating that the environment does not selectively stabilize the terminal-hydride $[Fe_4S_4(SCH_3)_4FeFe$-$H(CO)_3(CN)_2(dtma)]^{-3}$ isomer.

The next step in the catalytic cycle can be either protonation of dtma leading to **[H-dtmaH]$^{2-}$** (in which the Fe_4S_4 cluster remains in the +2 redox state; see Tables S3 and S4), or one-electron reduction of **[H-dtma]$^{3-}$** to give **[H-dtma]$^{4-}$** (Scheme 1). When considering the dtma-protonated forms **[tH-dtmaH]$^{2-}$** and **[μH-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 **[H-dtma]$^{3-}$** isomers (see Table 1). In addition, 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 kcal mol^{-1} to about 2 kcal mol^{-1} going from *Mod-1* to *Mod-4* (see Table 1).

When considering the reduced intermediate **[H-dtma]$^{4-}$**, the μ-hydride isomer is still thermodinamically more stable than the terminal-hydride form (**[μH-dtma]$^{4-}$** and **[tH-dtma]$^{4-}$**; Scheme 1 and Table 1), even though the energy gap is further decreased with respect to the values calculated for **[H-dtma]$^{3-}$** and **[H-dtmaH]$^{2-}$** isomers. It should be also noted that, when the entire H-cluster is considered (*Mod-2*, *Mod-3*, *Mod4*), in both **[μH-dtma]$^{4-}$** and **[tH-dtma]$^{4-}$** the unpaired electron is mainly localized on the Fe_4S_4 cluster (see Tables S3 and S4), which therefore can be formally assigned to the +1 redox state, analogously to models of the H-cluster in which dtma is replaced by pdt.[10] For the entire H-cluster models 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-dtma]$^{3-}$** rather than to the dinuclear model of **[H-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-dtma]$^{3-}$** , is significant as the energy difference decreases from about 11 kcal mol^{-1} to about 1 kcal mol^{-1} going from *Mod-1* to *Mod-4* (see Table 1).

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

Protonation of dtma in **[H-dtma]$^{4-}$**, leading to **[H-dtmaH]$^{3-}$**, is accompanied by electron transfer from the Fe_4S_4 to the $[2Fe]_H$ cluster. In fact, in **[H-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 S3 and S4). 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-dtmaH]$^{2-}$** to **[H-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-dtmaH]$^{3-}$**, the distance between the ammonium hydrogen 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 does not play any role, as in **[H-dtma]$^{3-}$**, or does play a minor but not crucial role, as in **[H-dtmaH]$^{2-}$** and **[H-dtma]$^{4-}$**, on the relative thermodynamic stability of $[2Fe]_H$-hydride intermediate species. These results, also corroborated by B3LYP data (see Supporting Information), lead to the conclusion that also in the enzyme active site terminal hydride species are 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, i.e. reaction with electrons and protons leading to H_2 formation must be considerably faster than terminal- to μ-hydride isomerization. In this scenario, as suggested also 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 Lys-237, which is strictly conserved in [FeFe]-hydrogenases, and forms a salt-bridge network involving the CN group coordinated to Fe_d (see Supporting Information). In fact, the electrostatic interaction between the positively charged side-chain of Lys-237 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 Lys-237 could be difficult to reproduce in bioinspired synthetic catalysts, mainly because CN ligands are generally avoided, since 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.

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- and reactive terminal-hydride species, as a function of the subcluster redox state and protonation state of the dtma chelating ligand. Analysis of simple dinuclear models of the H-cluster (Table 1, *Mod-1* column) reveals that the energy difference between μ- and terminal-hydride isomers decreases upon protonation of dtma and concomitant reduction of the binuclear cluster (results confirmed also using the B3LYP functional; see Supporting information). The implications of this remarkable observation for the reactivity of synthetic dinuclear clusters inspired to the [FeFe] hydrogenase active site are intriguing and worth exploring.

Keywords: hydrogenases · metal enzymes · broken symmetry calculations · H_2 production · QM/MM calculations

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[13] In the bimetallic complex (Mod-1), only an isomer with a semi-bridged CO is stable, both with the BP86 and B3LYP functionals. For

the entire 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 COs (rotated **[dtmaH]$^{3-}$,** or eclipsed [dtmaH']$^{3-}$ conformations) have been identified, with the latter slightly more stable than the former (1-2 kcal mol^{-1}). In the case of B3LYP only one isomer featuring a semi-bridged CO 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 $\mathbf{H_{red}}$ 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-dtma]$^{-3}$**), calculated for Mod-1, Mod-2 and Mod-3 models is exothermic by about 5 kcal mol^{-1}.

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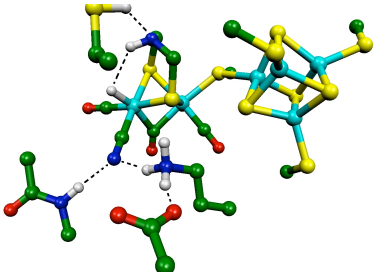

A step toward the disclosure of key rules for the design of bioinspired synthetic catalyst for H_2 production: how the environment of the [FeFe] hydrogenases catalytic cofactor affects its chemical properties