COMMUNICATIONS

Mechanism of H_2 metabolism on Fe-only hydrogenases

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The metabolism of hydrogen ($H_2 \leftrightarrow 2H^+ + 2e^-$) constitutes a central process in the global biological energy cycle. Among all the enzymes that can mediate this process, Fe-only hydrogenases are unique in their particular high reactivity. Recently, some important progresses have been achieved. Following our previous paper [Z.-P. Liu and P. Hu, J. Am. Chem. Soc. **124**, 5175 (2002)] that characterizes the individual redox state of the active site of Fe-only hydrogenase, in this work we have determined the feasible reaction pathways and energetics for the H_2 metabolism on the active site of Fe-only hydrogenases, using density functional theory. We show that H_2 metabolism possesses very low reaction barriers and a proximal base from a nearby protein plays an important role in H_2 metabolism. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1519252]

The metabolism of hydrogen ($H_2 \leftrightarrow 2H^+ + 2e^-$)[1–5] constitutes a central process in the global biological energy cycle. Among all the enzymes that can mediate this process, Fe-only hydrogenases are unique in their particular high reactivity.[2,5] In the last thirty years, tremendous efforts have been made experimentally, and some important progresses[6–13] have been achieved. However, a complete catalytic cycle involving H_2 oxidation/production has not been established to date. In this paper, we report feasible reaction pathways and energetics for the H_2 metabolism on the active site of Fe-only hydrogenases, using density functional theory (DFT). We show that H_2 metabolism possesses very low reaction barriers and a proximal base from a nearby protein plays an important role in H_2 metabolism.

Recently, some important advances in the understanding of structures[6–8] and redox states[8–10] of Fe-only hydrogenases were reported. The x-ray crystal structures show that the active center for H_2 metabolism in Fe-only hydrogenases consists of a novel *2Fe subunit* that is cysteine-S bridged to a [4Fe–4S] cubane[6–7] [Fig. 1(a)]. Both Fe ions in the 2Fe subunit are coordinated with biologically uncommon CO/CN-ligands [Fig. 1(a)].[5] For the sulfur-bridge linking two irons, a PDT chain ($-SCH_2CH_2CH_2S-$) was initially proposed in DdH (*D. desulfuricans*) by Nicolet *et al.*[6] Recently, they suggested that a DTN ($-SCH_2NHCH_2S-$) chain can be a better candidate.[10] More structural details on Fe-only hydrogenases can be found in previous work.[5,6,10,13] Theoretically, Dance carried out the first DFT calculations on possible reaction intermediates.[11] In his work, however, the model used for the 2Fe subunit was chemically different from the structures revealed later experimentally. Fan and Hall studied the heterolytic cleavage of H_2 using DFT.[12] Remarkably, their results showed that this step is highly reversible. In this work, we have carried out extensive DFT calculations aiming to obtain possible catalytic cycles and to understand the origin of the high reactivity of Fe-only hydrogenases for H_2 metabolism.

DFT with a generalized gradient approximation (GGA-PW91[14(a)]) was used in this study. Other calculation details are described in Refs. 14–17. A general consensus is that the reactivity of Fe-only hydrogenases for H_2 metabolism is determined by the 2Fe subunit while the [4Fe–4S] cluster is redox inactive.[5–7] In this study, the 2Fe subunit was modeled by $[(HS(CH_3))(CO)(CN^-)Fe^p(\mu\text{-DTN})(\mu\text{-CO})\times Fe^d(CO)(CN^-)(L)]^z$, as shown in Fig. 1(b), where Fe^d = the distal iron (relative to the [4Fe–4S] cluster), Fe^p = the proximal iron, L = the ligand bonding with the Fe^d at the transposition to μ-CO, z is the net charge in the system. This model has been utilized in our previous work, which showed very good agreement with the experiment.[13] A similar model was used by Hall and co-workers.[12,18]

By detailed comparison of possible candidates with experimental data, DFT calculations by Hall and co-workers[18] and us[13] have suggested that during H_2 metabolism the 2Fe subunit involves Fe(I)–Fe(I), Fe(II)–Fe(I), and Fe(II)–Fe(II) redox states. In addition, our analysis of the electronic structure of the 2Fe subunit showed that Fe(III)–Fe(III) or Fe(III)–Fe(II) oxidation states are not favored.[13] Recent synthetic work also suggested that in the 2Fe subunit low oxidation states are the catalytically active species.[19] Taking advantage of these findings, we explored the catalytic H_2 metabolism involving Fe(I)–Fe(I), Fe(II)–Fe(I), and Fe(II)–Fe(II) species. The low energy pathways are described as follows (Fig. 2).

Fe(II)–Fe(II) HYDRIDE FORMATION

Route 1: Starting from the fully reduced state, Fe(I)–Fe(I)(vacant) **1** (see the Fig. 2 caption), since the N of the DTN chain in **1** is a base, it can capture a proton from its surrounding forming **2**. Then complex **2** exothermally (0.1 eV) transforms to its isomer **2″**. Next the proton on the N

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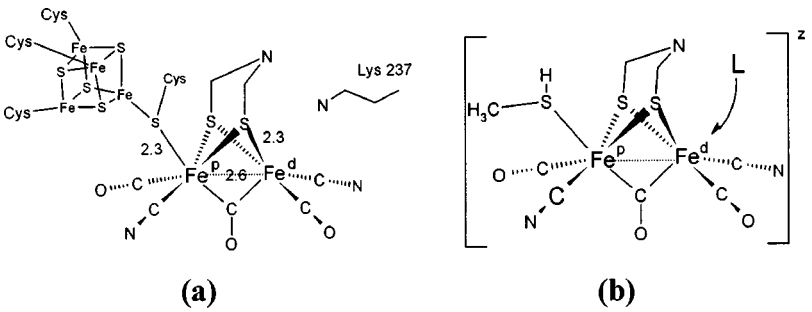

FIG. 1. (a) Active center of Fe-only hydrogenases as determined experimentally from *D. desulfurcicans*. (b) Calculation model used in this work.

moves to the Fe^d, yielding the Fe(II)–Fe(II)(H) **4**, through a transition state (TS) **3**. In this step, the 2Fe subunit is oxidized (two-electron oxidation) by the proton from a Fe(I)–Fe(I) complex to a Fe(II)–Fe(II) complex and the reaction barrier was determined to be 0.03 eV, as shown in Fig. 3.

Nicolet *et al.*[6] found in the x-ray crystal structure of DdH that the amino group of Lys 237 is 4.4 Å away from the Fe^d. It was speculated that this amino group may be a catalytic base in reactions.[6,7] To model its effect, we placed a NH_3 molecule near the Fe^d, which resulted in a second low energy route as follows. Route 2: Starting from structure **1′** (equivalent to **1**, see the caption of Fig. 2), the NH_3 picks up a proton forming NH_4^+ (**2′**). Then the proton from NH_4^+ directly moves to the Fe^d resulting in **4′** through the TS, **3′**, with a barrier of 0.10 eV. The energetic profile from **2′** to **4′** is shown in Fig. 3.

Fe(II)–Fe(II)(η-H_2) FORMATION

Route 3: The Fe(II)–Fe(II)(H) **4**, the highest oxidation state of the 2Fe subunit, may accept one electron (reduction)

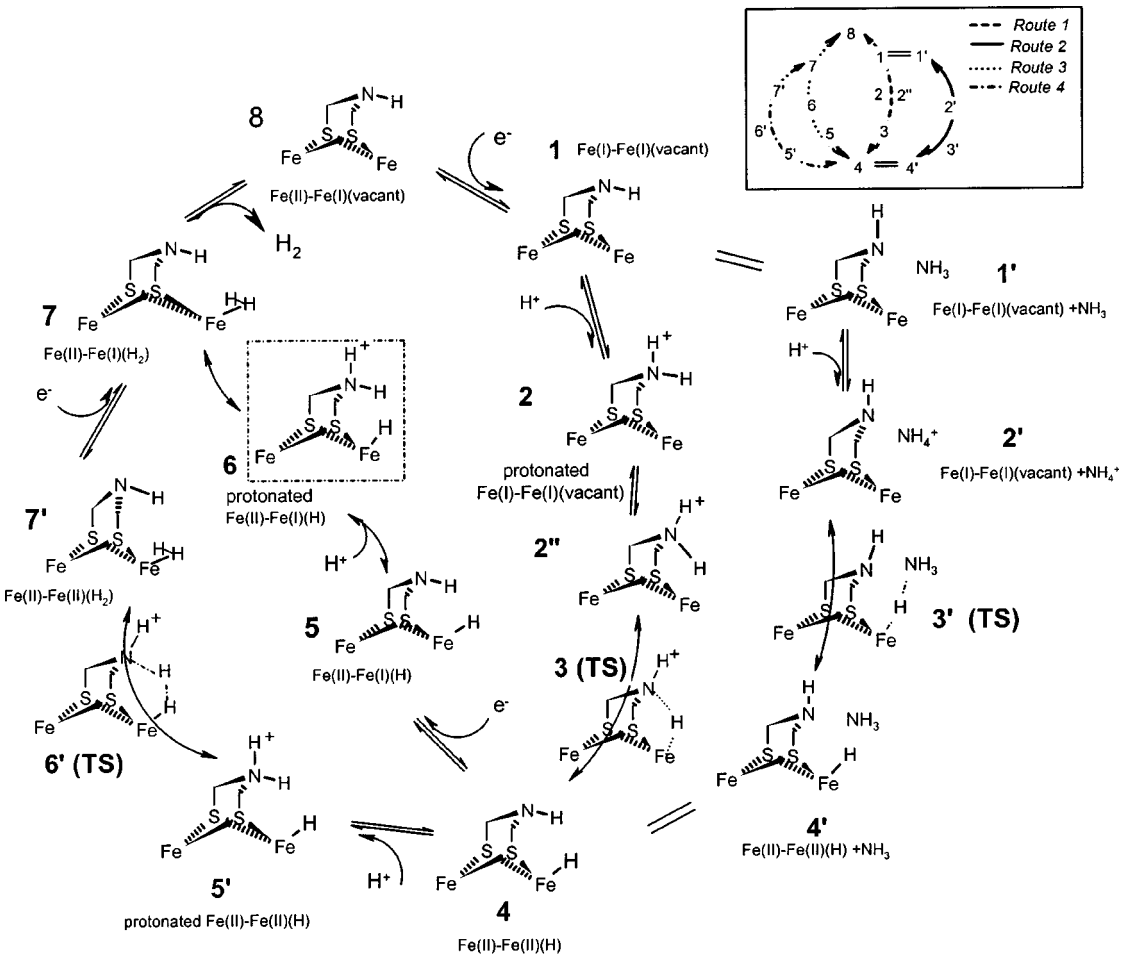

FIG. 2. Low energy catalytic cycles for H_2 metabolism calculated using DFT on the active center (2Fe subunit cluster) of Fe-only hydrogenases. The 2Fe subunit is modeled as described in the text and here is illustrated by a simplified structure. Each state is named and labeled beside the structure, and the ligand (L) that bonds with the Fe^d at the trans position to the bridging CO is emphasized. For example, the Fe(I)–Fe(I)(vacant) indicates that the L is absent; the Fe(II)–Fe(II)(H) means that the L is a H atom. Complex **4** and **4′** are equivalent, namely, they are exact same except in **4′** the NH_3 is used to model the Lys residue near the 2Fe subunit (the distance between the N in the NH_3 and the Fe^d is 4.3 Å in the optimized structure).[6] This is also true for complex **1** and **1′**. The dotted rectangle in complex 6 indicates the low stability of complex 6 (see text and Ref. 22). The insert at the upper corner presents the different reaction routes in the figure (also see text for details).

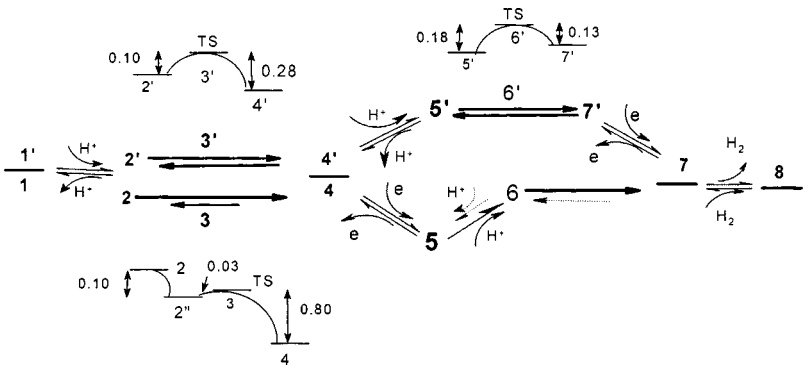

FIG. 3. Energetics in the determined catalytic cycles. Each intermediate state is labeled by the same number as in Fig. 2. The unit of energy is eV.

from other parts of the enzyme yielding the Fe(II)–Fe(I)(H) **5**. Then, the DTN chain of complex **5** accepts a proton from its environment to produce an intermediate **6**. The complex **6** is not stable: the optimization of complex **6** will rearrange **6** to the Fe(II)–Fe(I)(η-H_2) **7**.[20]

In parallel to route 3, an alterative route may exist. Route 4: Complex **4** can firstly capture a proton to form **5′**. Next, the proton moves to the Fe^d forming Fe(II)–Fe(II)(η-H_2) **7′** through the TS, **6′**, with a barrier of 0.18 eV, as shown in Fig. 3. In complex **7′** molecular H_2 is strongly bonded at the Fe^d (without H_2 at the Fe^d the Fe(II)–Fe(II)(vacant) transforms to hugely distorted structure (the 2Fe active site geometry is destroyed) after optimization due to the N in DTN chain being strongly attracted by the Fe^d). To release H_2, **7′** must be reduced to **7** (one-electron reduction), in which the (η-H_2)–Fe^d bond is very weak (0.09 eV). The reaction from **5′** to **7′** has been studied by Fan and Hall,[12] and our results here are consistent with theirs.

MOLECULAR H_2 RELEASE

In complex **7**, H_2 can be readily released, resulting in the Fe(II)–Fe(I) **8**. Complex **8** is a partially oxidized state and it may be further reduced (accepting one-electron) to finally complete the reaction cycle.[13] It should be emphasized the properties of the key intermediates in the reaction cycles investigated in this work (Fig. 2) such as geometrical structures, vibrational frequencies and EPR properties are consistent with the available experimental data (see Ref. 13 for details).

By examining the low energy routes, we found that each route has interesting characteristics with respect to the H_2 evolution and oxidation. The energetically favored cycle (cycle 1) for H_2 evolution from our calculation consists of route 1 and route 3, shown in Fig. 3. In this cycle, the highest barrier for H_2 evolution (from **2″** to **4**) is only 0.03 eV.[21] However, two key steps in this cycle are not favored for H_2 oxidation: The barrier from **4** to **2″** is too high (0.8 eV) in route 1 (Fig. 3); the reaction from **7** to **5** in route 3 cannot occur because the reverse reaction from **6** to **7** is a spontaneous process. This means that cycle 1 is not thermally feasible for H_2 oxidation at room temperature. For H_2 oxidation, the most energetically favored cycle is the one (cycle 2) that contains route 2 and route 4. The highest barrier for H_2 oxidation (from **4′** to **2′**) in this cycle is 0.28 eV (Fig. 3). It should be noted that which route is followed may also depend on the initial position of protons. If a proton initially

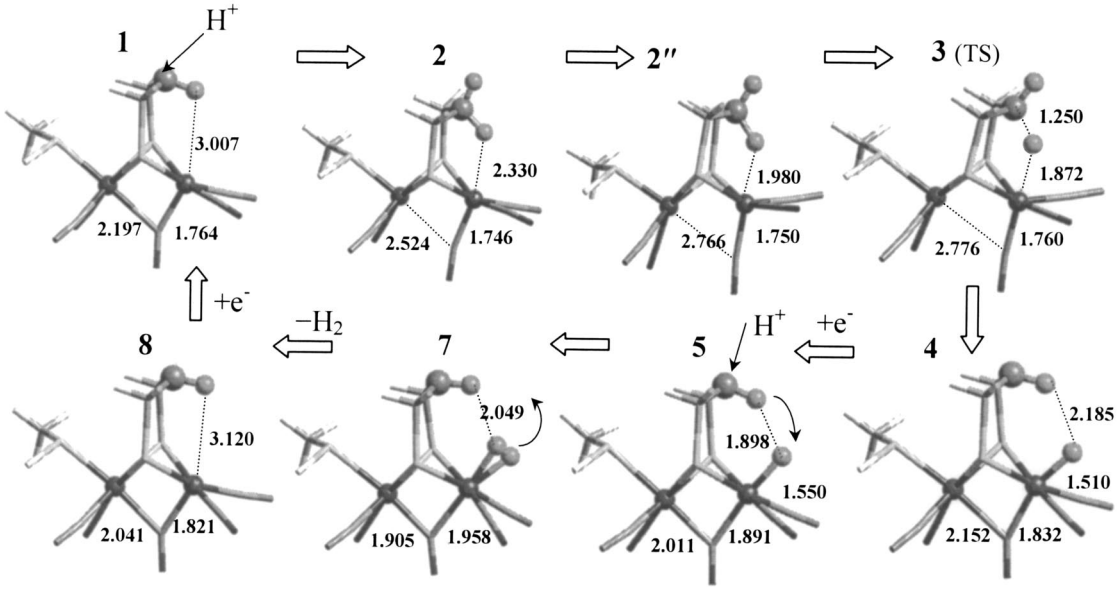

FIG. 4. Snapshots for the lowest energetic pathway of catalytic H_2 production, illustrated by optimized three-dimensional structures. Important atoms, e.g., Fe atoms, are shown by balls, and the others are presented by cylinders. Important distances (Å) in each state are labeled, which clearly shows the position variation of the bridging CO during the catalytic cycle.

locates on the Lys residue [Fig. 1(a)] not on the DTN, the reaction of H_2 evolution may follow route 2 rather than route 1, and vice versa.

A striking feature we found is that a rather strong catalytic base near the Fe^d is needed to facilitate the reaction from **4′** to **2′** (Fe^d–H bond breaking) in cycle 2, which may only be supplied by a nearby protein, such as Lys 237. We have tried many possible pathways for the reaction from **4** to **1** and found that no route will have a sufficiently low barrier except route 2.[22] This means that H_2 oxidation that follows cycle 2 relies strongly on a proximal base at the Fe^d. Without such a base, H_2 oxidation will not occur at room temperature. Thus, our results show that the proteins near a catalytic center are not simply ''spectators;'' they can directly participate in enzymatic catalysis.[20] It is interesting to note that a built-in base (DTN:-SCH_2NHCH_2S–) in the 2Fe subunit may not be essential for H_2 metabolism. Our calculation shows that the reactivity of Fe^d (the main reaction site) does not significantly change if the DTN chain is replaced by a PDT chain (–$SCH_2CH_2CH_2S$–). We expect that the same route as route 4 except the proton transfers through the Lys 237 (similar to route 2) not through the DTN exists. This will complete the catalytic cycle in conjunction with route 2 without the involvement of a DTN chain being a base.

Having determined the intermediates and the TSs in the completed catalytic cycles for $2H^+ + 2e^- \leftrightarrow H_2$, we are in a position to address why the 2Fe subunit is unique in its catalytic ability in addition to the features mentioned above. By examining the structure of the 2Fe subunit, we found that the bridging CO (CO_b) in the 2Fe subunit plays two important roles in H_2 metabolism. First, electronic structure analysis of the 2Fe subunit shows that the 2π orbital of the CO_b mixes with the e_g orbital of Fe (mainly the Fe^d) to form a frontier orbital,[13] which is quite delocalized. Through the CO_b, it extends from the Fe^d to the Fe^p, and also to the cysteine-S at the Fe^p. Therefore, this orbital is expected to facilitate electron flow to and from the Fe^d. Second, during the reactions the CO_b varies its position relative to the Fe^d and the Fe^p, which efficiently facilitate bond making/breaking occurring at the Fe^d, as shown in Fig. 4. For example, in the reaction from **4** to **2**, the Fe^d–H bond breaking is accompanied by the CO_b shifting to the Fe^d, while from **8** to **7** the H_2 uptake at the Fe^d is concomitant with the CO_b shifting away from the Fe^d. Thus the CO_b plays a ''gate'' role to determine the coordination at the Fe^d.

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[15] The calculation methods used in this work are the same as that described in our previous paper, Ref. 13. The program used is CASTEP (see Ref. 23), which implements the DFT total energy calculation with plane wave basis set and ultrasoft pseudopotentials [see Ref. 14(b)]. The transition states are searched with constrained minimization technique (see Refs. 16, 17). The accuracy of the current DFT total energy calculation, in particular for the calculation of reaction barriers (the error of the barrier is normally within 0.1 eV), has been demonstrated previously (see Refs. 13, 16, 17 and the references therein). This calculation method has been used to study biological systems, e.g., nitrogen fixation on MoFe6S9 (the active site of nitrogenase) by Norskov group [T. H. Rod, B. Hammer, and J. K. Norskov, Phys. Rev. Lett. **82**, 4054 (1999)].
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[20] The nearby protein may also help to stabilize the reaction intermediates during the catalytic reaction through the hydrogen-bonding with the 2Fe subunit. For example, complex **6** is not a stable species. Once formed, the H^+ can spontaneously move to the Fe^d to produce complex **7**. However, with H-bonding that was modeled by placing one H_2O near the N in the DTN, complex **6** can be stable.
[21] Here the barriers for the proton and electron transfer are not considered since these processes are well-addressed and are believed to be efficient in the enzymatic catalysis (see Refs. 1–7).
[22] Our calculations further imply that the Fe^d–H bond breaking (e.g., from **4′** to **2′**) is sensitive to the pK_a of this proximal base. By replacing the NH_3 (route 2) with a H_2O, we found that the barrier for the Fe^d–H bond breaking increases to 0.52 eV.
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