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A Functional Model of [Fe]-Hydrogenase

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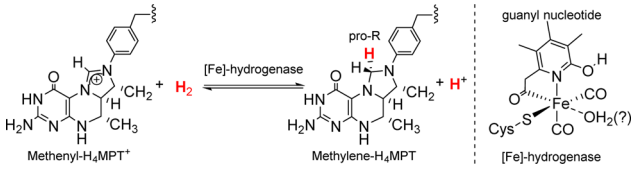
Supporting Information

ABSTRACT: [Fe]-Hydrogenase catalyzes the hydrogenation of a biological substrate via the heterolytic splitting of molecular hydrogen. While many synthetic models of [Fe]-hydrogenase have been prepared, none yet are capable of activating H_2 on their own. Here, we report the first Fe-based functional mimic of the active site of [Fe]-hydrogenase, which was developed based on a mechanistic understanding. The activity of this iron model complex is enabled by its unique ligand environment, consisting of biomimetic pyridinylacyl and carbonyl ligands, as well as a bioinspired diphosphine ligand with a pendant amine moiety. The model complex activates H_2 and mediates hydrogenation of an aldehyde.

As the third class of hydrogenases, [Fe]-hydrogenase catalyzes the reduction of methenyl-tetrahydromethanopterin (methenyl-H_4MPT$^+$) with H_2 to form methylene-tetrahydromethanopterin (methylene-H_4MPT) and H^+ (Scheme 1, left).[1] This

Scheme 1. Hydride-Transfer Reaction Catalyzed by [Fe]-Hydrogenase and Proposed Active Site



is an intermediate step in the reduction of CO_2 to methane mediated by some methanogenes. [Fe]-hydrogenase has a single Fe-containing active site in which the Fe ion is coordinated to a cysteine sulfur atom, two cis-oriented CO ligands, the acyl carbon, and pyridinyl nitrogen atoms of a guanylylpyridinol ligand, and possibly a water molecule (Scheme 1, right).[2]

The unique structure and function of [Fe]-hydrogenase have motivated the synthesis of a number of small-molecular mimics,[3] however, until now none of these mimics was successful in splitting H_2.[4] Meyer et al. reported an alternative approach in which the methenyl-H_4MPT$^+$ substrate was modeled using an imidazolium salt.[5] The resulting frustrated Lewis pair comprised of the imidazolium salt along with a polymeric Ru metalate did split H_2. Nevertheless, an Fe-based functional mimic of [Fe]-hydrogenase remains elusive. Herein, we report the first example of an Fe-based functional mimic capable of heterolytically cleaving H_2. The model is designed based on experimental and computational mechanistic insights.

Recently, we reported model complexes **1** and **2**, which faithfully reproduce the first coordination sphere of the Fe center in [Fe]-hydrogenase, and in the case of **2**, even the hydroxy group at the second coordination sphere (Figure 1).[6] However, both **1**

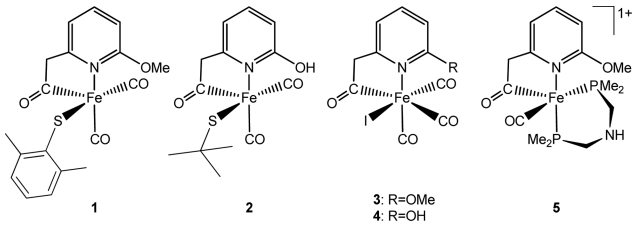


Figure 1. Previous model complexes of [Fe]-hydrogenase.

and **2** fail to activate H_2, despite possessing similar electronic properties to the [Fe]-hydrogenase active site as judged from infrared (IR) spectroscopic data. Semisynthetic [Fe]-hydrogenases were reconstituted using the apoenzyme along with complexes **3** and **4**,[7] the precursors to **1** and **2**, respectively. The enzyme reconstituted with **4** is active, while the enzyme reconstituted with **3** is inactive.[8] This result highlighted the essential role of the hydroxy group at the second coordination sphere. DFT computations indicated that deprotonating the hydroxy group allowed it to serve as an internal base for heterolytic H_2 splitting.[9] When deprotonation is not possible (as in **1** and **3**), and the cysteine thiolate is forced to act as the proton acceptor, the energetic barrier associated with H_2 splitting becomes inaccessible, and correspondingly, no activity is observed.[8] These results suggest that to achieve H_2 activation outside the enzyme, model complexes of [Fe]-hydrogenase must have a competent internal base.

According to this mechanistic insight, deprotonation of the hydroxy group in **2** and **4** should lead to model complexes capable of splitting H_2. Unfortunately, treatment of **2** and **4** with a base such as KO^{t}Bu, Et_3N, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) led to decomposition of the complexes. Thus, alternative approaches for introducing an internal base must be found. Recently, P^RNP^R and P_2N_2 ligands were shown to induce remarkable activity in functional models of [FeFe]-hydrogenase thanks to the pendant nitrogen's ability to serve as a proton

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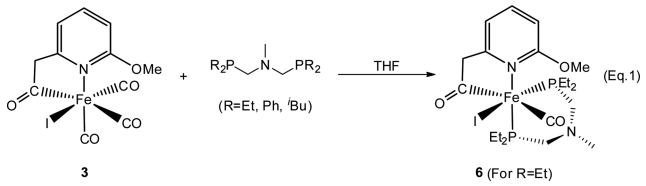
acceptor.[10] DFT computations of an [Fe]-hydrogenase mimic bearing a $P^{Me}NP^{Me}$ ligand showed that hypothetical complex **5** indeed splits H_2 with an accompanying barrier of only about 5 kcal/mol and could also hydrogenate aldehyde.[11] Guided by these computational findings, we sought to prepare analogous model complexes.

Reactions of complex **3** with several P^RNP^R ligands were conducted, however, only for $Et_2PCH_2NMeCH_2PEt_2$ (PNP) was an isolable complex **6** obtained, albeit with a low yield (eq 1). The



solid-state molecular structure, determined by X-ray diffraction, confirmed that the PNP ligand replaced two of the three CO ligands in **3** (Figure 2). Note that the remaining CO ligand

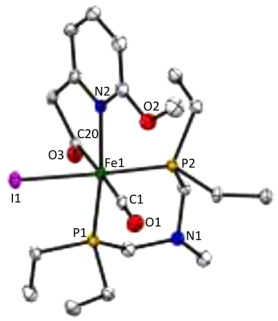


Figure 2. Solid-state structure of complex 6. The thermal ellipsoids are displayed at 50% probability. Selected bond lengths [Å] and angles [°]: Fe–I, 2.7169(5); Fe–C1, 1.830(4); Fe–C20, 1.982(4); Fe–N2, 2.070(3); Fe–P1, 2.2382(10); Fe–P2, 2.1990(10); C1–O1, 1.147(4); C20–O3, 1.222(4); I–Fe–P2, 176.28(3); I–Fe–C1, 86.74(10); N2–Fe–P1, 168.80(9); N2–Fe–C20, 81.77(13); P1–Fe–P2, 91.37(4).

occupies the trans position, while the iodide ligand remained at the cis position, relative to the acyl carbon atom. Compared to complex **3**, the Fe–I bond is elongated by 0.06 Å, and the Fe-CO bond is shortened by 0.07 Å in complex **6**. The CO vibrational frequency is 1945 cm^{-1} in the solid-state IR spectrum of **6**, which aligns closely with one of the two CO vibrational frequencies of [Fe]-hydrogenase (1944 and 2011 cm^{-1}).[12] The chemical shift of the PNP ligand is observed at 57.7 and 42.6 ppm in the ^{31}P NMR spectrum.

A H_2/D_2 exchange assay was employed to determine if **6** is capable of activating H_2. H_2 (8 bar) and D_2 (18 bar) were added to a solution of **6** in D_8-THF, and the reaction was monitored by NMR spectroscopy. HD was immediately (<5 min) observed in the ^1H NMR spectrum (Figure 3). The formation of HD was also immediate (<5 min) with reduced pressure, i.e., 1 bar H_2 and 2 bar D_2. This rapid H_2/D_2 exchange is consistent with a facile and reversible cleavage of H_2 and D_2 by complex **6**. HD was formed when H_2 was added to a solution containing both **6** and D1-acetic acid, supporting a heterolytic H_2 cleavage process. Scheme 2 shows the proposed mechanism for the exchange. The reaction proceeds by substitution of iodide by H_2, possibly through a dissociative mechanism (**6** to **8** via **7** and **7′**). Bifunctional activation of H_2 by the Fe ion and the pendant amine of the PNP ligand then yields the hydride complex **9** containing a

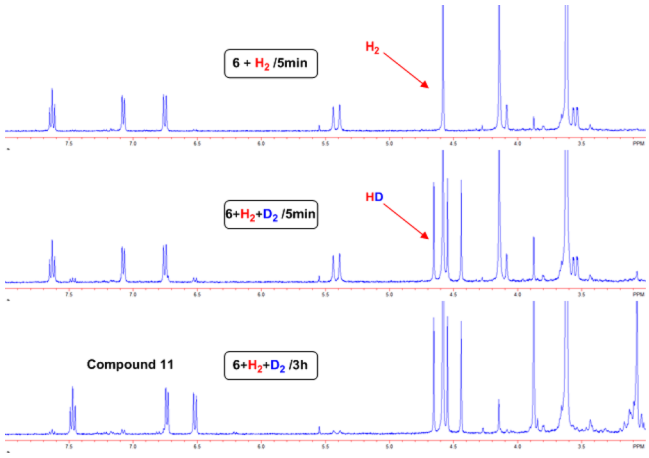
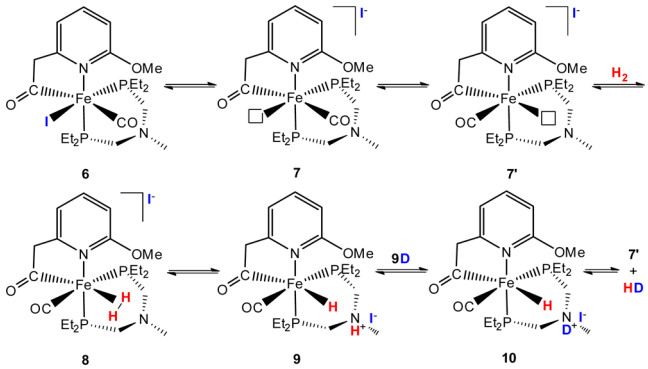


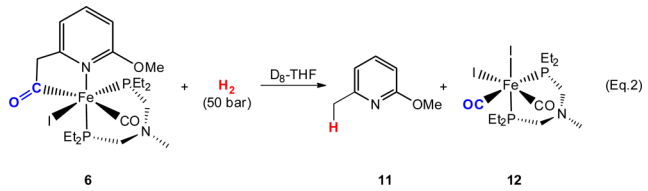
Figure 3. Reaction of complex **6** with H_2/D_2.

Scheme 2. Proposed Mechanism for the H/D Exchange



trialkylammonium moiety. Exchange of the proton with the deuterium of the analogous complex **9D**, produced from the reaction of D_2 with **6**, gives complex **10**. Recombination of the hydride ligand with the deuterium ion leads to formation of HD.

The reaction of **6** with H_2 (50 bar) in D_8-THF was then monitored by NMR spectroscopy. However, no signals corresponding to species **9** could be detected, suggesting that **9** is an unstable intermediate. Conducting the reaction for 5 h resulted in two isolatable products identified from the reaction mixture: 2-methoxyl-6-methyl-pyridine (**11**, > 95% NMR yield) and complex [Fe(PNP) $(CO)_2I_2$] (**12**; 40% yield) (eq 2).[13]



When H_2 was replaced by D_2 in the reaction, a deuterated 2-methoxyl-6-methyl-pyridine was obtained. The solid-state molecular structure of **12** was determined by X-ray crystallography (see Supporting Information (SI)). The two CO ligands are cis to one another, as are the two iodide ligands. The CO vibrational frequencies are observed at 2023 and 1969 cm^{-1} in the IR spectrum of **12**, which are slightly higher than those of [Fe]-hydrogenase. In the ^{31}P NMR spectrum, the signals of the phosphine ligands are observed at 46.9 and 6.3 ppm. The formation mechanism of **11** and **12** is unclear and may involve

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multiple pathways. One possibility involves protonation of **6** by the unstable intermediate **9** (for details, see SI).

As dissociation of I^- from **6** to form **7** is proposed as a key step in Scheme 2, several experiments were conducted to probe the nature of this step.[14] The H_2/D_2 exchange was found to be slower (for comparison of HD formation rate, see the SI) in the presence of excess KI or $^nBu_4NI$, providing support for the proposed dissociation of I^- from **6**. The IR and NMR spectra of **6** in solution were carefully analyzed for the presence of **7**, however, no signal was detected at the limit of resolution. This result suggests that the dissociation of I^- is thermodynamically uphill. Complex **6** was treated with a halide abstractor such as $AgBF_4$ or NaBARF (BARF = tetrakis[3,5-bis(trifluoromethyl)phenyl]borate), which led only to unidentifiable decomposition products.

The H_2/D_2 exchange mediated by **6** was followed by IR and NMR, however, none of the proposed intermediates **7**−**10** were detected. **6** was the sole observable Fe-containing species, suggesting that each of the intermediates are energetically less stable than **6**. To provide energetic estimates involved in H_2/D_2 exchange reaction, DFT computations were conducted. The computed free energy profile (at the B3LYP[15]-dDsC[16]/TZ2P//M06[17]/def2-SVP level including solvation corrections in implicit THF using COSMO-RS,[18] see SI for full computational details) are presented in Figure 4. The dissociation of iodide from **6** is

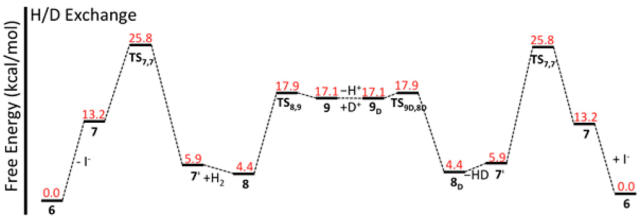
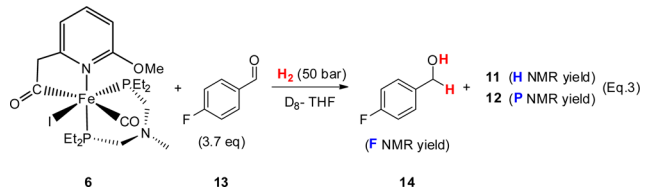


Figure 4. Free energy profile of H_2/D_2 exchange process.

endergonic by over 13 kcal/mol, though this value might be overestimated due to the difficulties of describing the solvation of monovalent anions in implicit solvent.[19] $\mathbf{TS_{7,7'}}$, the transition state associated with isomerization of the CO group from a trans to cis position (relative to the acylpyridinyl moiety) marks the highest point on the potential energy surface (25.8 kcal/mol). H_2 easily coordinates to **7′** in an exergonic process and can then be heterolytically cleaved by proceeding through $\mathbf{TS_{8,9}}$ with a cost of ∼13.5 kcal/mol from **8**. The resulting product, **9**, is relatively unstable and would quickly reform H_2 (**9** → **8**) or undergo HD exchange (**9** → **9D** → **7′**). Considering the inherent uncertainty accompanying any DFT computation, our highest reaction barrier is in reasonable agreement with the experimental results. According to the computations, all intermediates **7**−**10** are less stable than **6**, which explains why no evidence of their presence is observed during the catalysis. For an alternative mechanistic pathway involving dissociation of a CO rather than an I^- ligand, the resulting free energy profile was found to be considerably less favorable, with relative free energies exceeding 40 kcal/mol for H_2 cleavage.

Eq 2 indicates that during the reaction of model complex **6** with H_2, the complex tends to decompose to Fe PNP complexes lacking the biomimetic acylpyridinyl ligand. To test whether the hydride ligand in the intermediate **9** could be transferred to an unsaturated organic substrate before the decomposition of **6**, the hydrogenation of *para*-fluorobenzaldehyde (**13**) was conducted in the presence of **6** (eq 3).[13] This aldehyde was chosen because the reactions could be easily followed by ^{19}F NMR spectroscopy.



To our delight, hydrogenation of the aldehyde was observed. The yield of alcohol (**14**) was 82% after 2h and 113% in 4h relative to the amount of **6**. The rate of hydrogenation was slower when the reaction was conducted in the presence of excess KI, which is consistent with I^- dissociation being an important step for H_2 activation (for details, see SI). Deuterated alcohol (**14**) was formed when D_2 was used in place of H_2 in the reaction. The low turnover numbers are partially due to the decomposition of **6** under reaction conditions, as described above. Indeed, both **11** and **12** were observed during the reaction as side products.

To confirm that hydrogenation of **13** was mediated by **6** rather than **12**, the reaction profile of the hydrogenation of **13** was determined by ^{31}P, ^{19}F, and ^1H NMR spectroscopy (Figure 5).

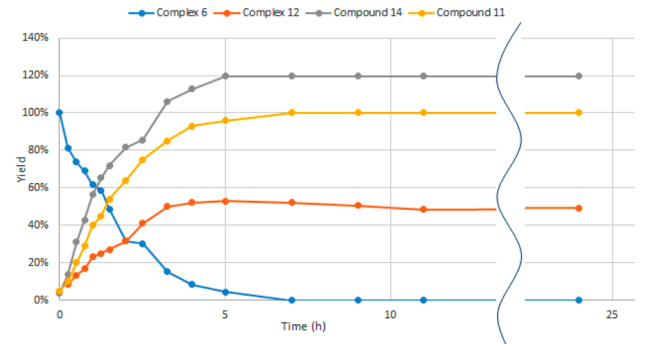


Figure 5. Reaction of complex **6** with aldehyde under H_2 gas.

The hydrogenation was rapid initially, but slowed as **6** decomposed into **11** and **12**. When all of **6** was decomposed, no further hydrogenation occurred. The reaction profile suggests **6** is responsible for hydrogenation. The isolated complex **12** was then tested for hydrogenation of **13**, but no hydrogenation was found. These results further confirm the role of **6** in the hydrogenation of **13**.

Scheme 3 depicts a hypothetical and simplified reaction sequence accounting for the outcomes of the H_2/D_2 exchange and hydrogenation reactions described here. Iodide dissociation from model complex **6** and isomerization give the 5-coordinate intermediate **7′**. Binding and heterolytic splitting of H_2 by **7′** gives the hydride complex **9**, which is the common intermediate for H_2/D_2 exchange and hydrogenation. The proposed mechanism for the H_2/D_2 exchange is depicted in Scheme 2. For the hydrogenation, the reaction might proceed stepwise or by a concerted proton-hydride transfer.

In conclusion, we report the first iron-based functional model of [Fe]-hydrogenase, designed based upon mechanistic insights. This model complex (**6**) activates H_2 in a heterolytic manner and mediates hydrogenation of an aldehyde molecule. The efficiency of the complex in catalytic hydrogenation is limited by the decomposition of complex **6**, resulting in decarbonylative dissociation of the acylpyridinyl ligand. Future work will focus on improving the stability of the complex, which may lead to more efficient hydrogenation catalysts. Moreover, structure−activity studies employing other ligands containing internal bases will be

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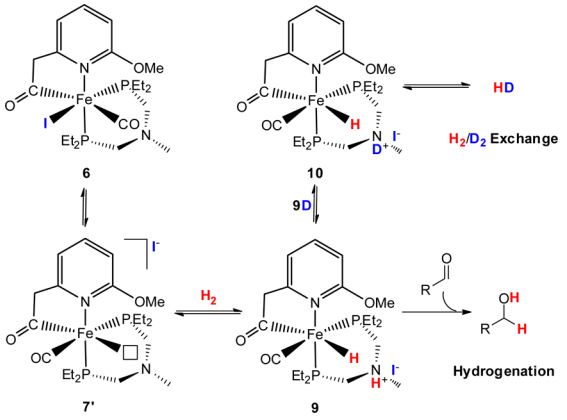
Scheme 3. A Proposed Mechanism for Both H_2/D_2 Exchange and Hydrogenation



conducted. Notwithstanding, this work demonstrates, for the first time, that an Fe-based model of [Fe]-hydrogenase capably activates H_2 outside the enzyme and without its biological substrate, H_4MPT^+.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b12095.

Experimental details and data (PDF)
Crystallographic data (CIF)
Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

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(13) The equation is not balanced because not all the products were identified in the reaction. The equation is meant to be approximate. For eq 2, we suspect that two molecules of **6** react with H_2 to give two molecules of **11**, one molecule of **12**, and an undetected Fe(0) species.

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