Biomimetic Chemistry

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Reversible Protonation of a Thiolate Ligand in an [Fe]-Hydrogenase Model Complex**

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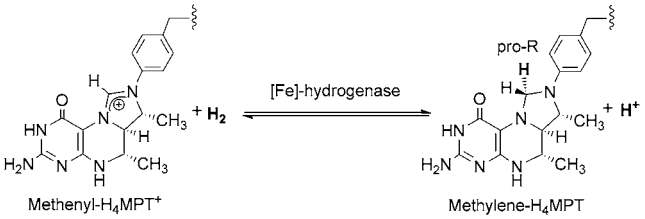
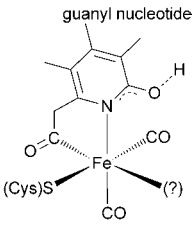
[Fe]-hydrogenase is involved in the methanogenic pathway of the reduction of CO_2 to methane.[1–3] In the presence of methenyltetrahydromethanopterin (methenyl-H_4MPT$^+$), this enzyme catalyzes the heterolytic cleavage of H_2 (Scheme 1).[4]



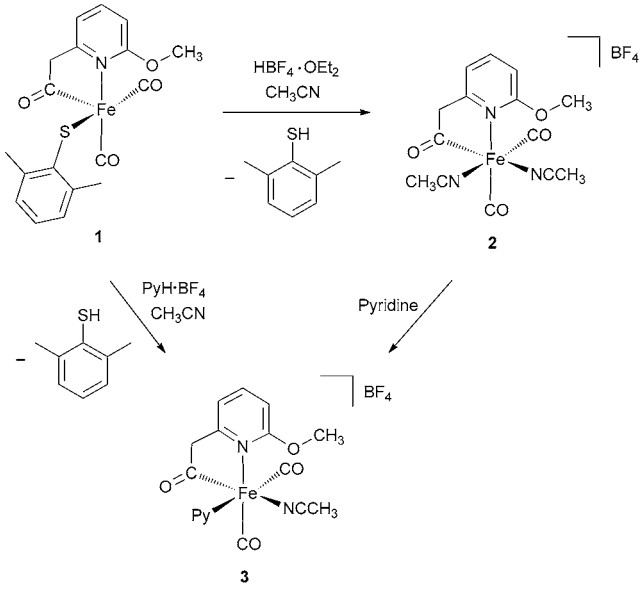
Scheme 1. Enzymatic function of [Fe]-hydrogenase. The reaction has a $\Delta G^{o\prime}$ of -5.5 kJ mol^{-1}.

The resulting hydride ion is transferred to methenyl-H_4MPT$^+$ to form methylenetetrahydromethanopterin (methylene-H_4MPT). A proton is also formed from this reaction, and it is shown that this proton exchanges quickly with protons of the bulk solvent (water).[4] The immediate proton acceptor is unknown. The cysteine thiolate ligand (Cys176), the pyridonal hydroxy group in the secondary coordination sphere, and even the acyl ligand of the Fe center (Scheme 2), might function as the internal base. Herein we describe the protonation reactions of a model complex of [Fe]-hydrogenase. The study indicates that the cysteine thiolate ligand is a viable proton acceptor for [Fe]-hydrogenase.



Scheme 2. The proposed active site of [Fe]-hydrogenase. The sixth coordination site might be empty, or occupied by a water molecule. Cys = cysteine.

According to current spectroscopic[5–12] and crystallographic data,[13–16] the active site of [Fe]-hydrogenase consists of a mononuclear Fe^{II} complex. The Fe ion is ligated by two *cis*-CO, a cysteine sulfur atom (Cys176), and the pyridyl and acyl donor atoms of a guanylylpyridinol cofactor (Scheme 2). The coordination site *trans* to the acyl ligand is proposed as the H_2-binding site. It is unclear whether this site is vacant or occupied by a H_2O molecule in the resting state. Based on this information, a number of model complexes have been prepared.[17–31] We recently synthesized a five-coordinate Fe^{II} model complex (**1**, Scheme 3).[31] This complex provides a unique platform for reactivity studies, including those of protonation reactions.



Scheme 3. Reactivity of **1** with acids in CH_3CN. Py = pyridine.

Reaction of the red compound **1** with $HBF_4{\cdot}Et_2O$ in CH_3CN produced a free thiol molecule and a yellow compound **2** (Scheme 3). The ^{1}H NMR spectrum of **2** exhibits three signals at 7.98, 7.19 and 6.98 ppm for the pyridyl ring, two doublets at 4.72 and 3.89 ppm for the diastereotopic methylene hydrogen atoms, one singlet at 4.01 ppm for the methoxy group, and one singlet at 1.96 ppm for the coordinated CH_3CN molecule.[32] The integration of the signals from the CH_3CN ligands indicates that the Fe center is ligated by two molecules of CH_3CN. In the ^{13}C NMR spectrum of **2**, one acyl carbon ($\delta = 260.6$ ppm) and two carbonyl carbon atoms

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($\delta = 208.4$ and 207.1 ppm) can be identified. The IR spectrum of **2** shows two intense absorption bands indicating two *cis*-oriented CO ligands (Table 1). Thus, according to the NMR and IR spectroscopy, complex **2** has the formula of [(2-CH_2CO-6-$MeOC_5H_3N$)$Fe(CO)_2(CH_3CN)_2]BF_4$ (Scheme 3). This formulation is confirmed by elemental analysis.[32] Unfortunately, we were not able to obtain single crystals of **2**.

Table 1: Selected IR spectroscopy data.

Complex	ν_{CO} [cm^{-1}]
1[a]	2013, 1950
2	2059, 1997
3	2042, 1979
[Fe]-hydrogenase[b]	2011, 1944

[a] Data from Ref. [31]. [b] Data from Ref.[6].

Reaction of **1** with pyridinium tetrafluoroborate ($PyH{\cdot}BF_4$) in CH_3CN did not lead to **2**, but rather to [(6-$MeOC_5H_3N$-2-CH_2CO)$Fe(CO)_2(CH_3CN)(C_6H_5N)]BF_4$ (**3**; Scheme 3). Compound **3** was also prepared by reaction of **2** with pyridine in CH_3CN (Scheme 3). Interestingly, in the latter reaction, only one CH_3CN ligand was replaced by pyridine, even when an excess amount of pyridine was used. Single crystals suitable for an X-ray diffraction study were obtained by diffusion of ether/pentane into a solution of **3** in CH_3CN.

The crystal structure of complex cation in **3** is shown in Figure 1.[33] The coordination geometry of the Fe center is octahedral. The acylmethylpyridinyl moiety coordinates by the pyridyl nitrogen and acyl carbon donors, giving rise to a five-membered metallacycle. The two CO ligands are *cis* to one another; they are both *cis* to the acyl ligand as well. This arrangement reflects the strong trans influence of CO and acyl ligands. The pyridine ligand sits on the position *trans* to one of the CO ligands, which was originally occupied by the thiolate ligand. The CH_3CN ligand occupies the position *trans* to the acyl ligand.

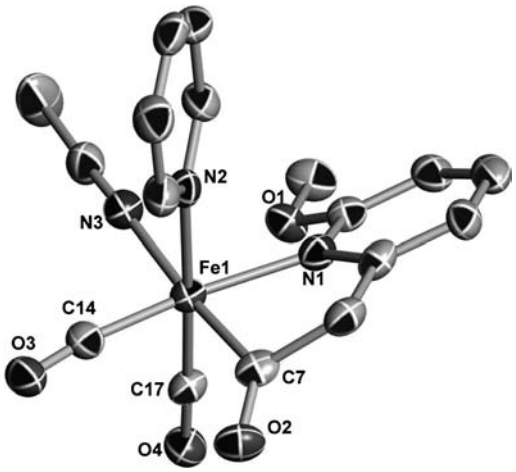
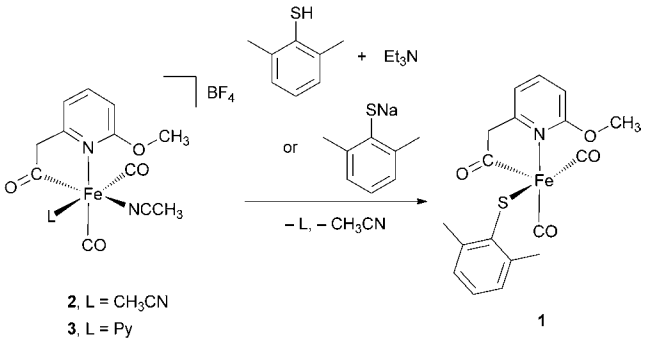


Figure 1. Solid-state molecular structure of the complex cation in compound **3**. There are two independent molecules in each asymmetric unit; only one of them is shown. Thermal ellipsoids are set at 30% probability. Selected bond lengths [Å] and angles [°]: Fe1–N1 2.041(6), Fe1–N2 2.028(6), Fe1–N3 2.028(7), Fe1–C7 1.954(7), Fe1–C14 1.785(8), Fe1–C17 1.770(8), C7–O2 1.200(9), C14–O3 1.150(9), C17–O4 1.147(9), C14-Fe1-C17 88.6(3).

The protonation and decoordination of the thiolate ligand in **1** are reversible. Treatment of **2** and **3** with a mixture of NEt_3 and $HS(2,6\text{-}Me_2C_6H_3)$ in CH_3CN regenerated complex **1** (Scheme 4). No reaction took place between NEt_3 and $HS(2,6\text{-}Me_2C_6H_3)$ alone in CH_3CN. Thus, $HS(2,6\text{-}Me_2C_6H_3)$ must first coordinate to the Fe ions in **2** and **3** to form Fe-thiol species as intermediates. Upon binding of sulfur to Fe, the thiol proton became more acidic and could be deprotonated by Et_3N to give the thiolate complex **1**. It is noteworthy that the thiolate ligand enforces a five-coordinate geometry on the Fe center, expelling the solvent molecule that originally occupies the position *trans* to the acyl ligand. Complex **1** was also produced by reaction of **2** with $2,6\text{-}Me_2C_6H_3SNa$.



Scheme 4. Regeneration of **1** from reactions of **2** and **3** with a thiol ligand under basic conditions.

The reactivity of model compounds **1**–**3** provides suggestions for the catalytic mechanism of [Fe]-hydrogenase. It infers that the Cys176 thiolate ligand in the enzyme can be the immediate proton acceptor after H_2 splitting. In the model system, the resulting thiol molecule is too weak a ligand for the Fe ion, so it is replaced by a solvent molecule. A similar ligand substitution reaction may occur in the enzyme after Cys176 is protonated. It is however possible that the protein scaffold enforces the sulfur coordination. In any case, the thiol proton needs to be transferred to a relay base to regenerate the thiolate ligand, which will restore the active site. This proton transfer step is fast according to an earlier study.[4] In the model study, the relay base is Et_3N. For the enzyme, the relay base could be the pyridinol group or the His14 group. It was reported that a His14 exchange reduced the activity of the wild enzyme to less than 1%.[16] Speculatively, the important role of His14 might be to regenerate the thiolate ligand and thus the active site.

The IR spectra of both **2** and **3** show two intense ν_{CO} absorption bands (2059 and 1997 cm^{-1} for **2**; 2042 and 1979 cm^{-1} for **3**), consistent with the presence of two *cis*-CO groups (Table 1). Compared to complex **1** and [Fe]-hydrogenase, the averaged ν_{CO} stretching frequencies of **2** and **3** are significantly higher (> 30 cm^{-1}). This shift reflects the charge difference among model compounds **1**–**3**. Cationic Fe complexes in **2** and **3** have significantly higher ν_{CO} than the neutral

Fe complex **1**. The IR data in turn suggest that the Fe complex in the active site of [Fe]-hydrogenase is likely neutral, because its ν_{CO} is very close to that of **1**, but not those of **2** and **3** (Table 1).

In summary, the thiolate ligand in complex **1**, a unique small-molecule mimic of the active site of [Fe]-hydrogenase, can be reversibly protonated. This study suggests that the Cys176 ligand in [Fe]-hydrogenase can serve as an internal base to accept the proton from H_2 before delivering it to the bulk solvent. This function is indispensible for catalytic turnover. In synthetic chemistry, thiolate ligands acting as proton acceptors are well-known.[34,35] In [NiFe]-hydrogenase, a thiolate ligand is proposed as the internal base that promotes heterolytic H_2 splitting.[36] Rauchfuss et al. found earlier that a Fe-acyl model complex of [Fe]-hydrogenase, $Fe(SPh)(Ph_2PC_6H_4CO)(CO)_3$, could react with $H(OEt_2)$-$BAr^F{}_4$ ($ArF = 3,5\text{-}(CF_3)_2C_6H_3$) at −30 °C in CH_2Cl_2.[28] If NEt_3 was added into the resulting solution, the starting compound was reformed. However, no protonation product could be identified, so the proton acceptor was not clear.[28] The current work, therefore, establishes for the first time, the protonation chemistry of [Fe]-hydrogenase models. It is significant that the thiolate ligand is preferentially protonated in the presence of an acyl ligand which is commonly conceived as highly basic and reactive. The chemistry offers reference for mechanistic proposals of [Fe]-hydrogenase activity.

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[32] See the Supporting Information.
[33] CCDC 851252 (**3**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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