Hydrogenase

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A Five-Coordinate Iron Center in the Active Site of [Fe]-Hydrogenase: Hints from a Model Study**

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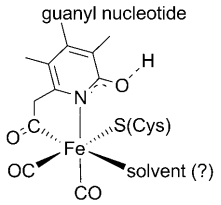
[Fe]-hydrogenase catalyzes the reversible reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT^+) with H_2 to form methylenetetrahydromethanopterin (methylene-H_4MPT) and H^+.[1–3] This reaction is an intermediary step in the reduction of CO_2 to methane by methanogens grown under nickel-limiting conditions. Crystallographic[4–7] and spectroscopic[8–14] analyses of the wild-type enzyme and its mutants have revealed a unique and intriguing active site: a single iron ion is coordinated to a cysteine sulfur atom, two *cis* CO ligands, and a bidentate pyridone cofactor with pyridinyl nitrogen and acyl carbon donor atoms (Scheme 1). There is still uncertainty in the exact coordination number and geometry of the Fe center. Current data suggest that the Fe center could be either five-coordinate (square-pyramidal) or six-coordinate (octahedral).[5,7,14] In the octahedral model, the sixth ligand is assumed to be a labile solvent molecule. In CO- and CN^--inhibited [Fe]-hydrogenase, the sixth position is occupied by CO and CN^-, respectively.[9,10]



Scheme 1. The proposed active site of [Fe]-hydrogenase.

Regardless whether the resting state of the iron center is five- or six-coordinate, the active species during catalysis is proposed to be five-coordinate.[7,14] A number of small-molecule mimics of [Fe]-hydrogenase have been reported.[15–25] To date, all of the advanced models that contain iron acyl or carbamoyl moieties[17,19,20,23,24] are six-coordinate. Herein we describe the synthesis, structure, and reactivity of the first five-coordinate model complex reproducing the coordination sphere of the Fe ion in [Fe]-hydrogenase. This work provides an interesting chemical precedent for the enzymatic study of [Fe]-hydrogenase.

We previously prepared an Fe^{II} tris(carbonyl) complex [(2-CH_2CO-6-$MeOC_5H_3N$)Fe(CO)$_3$I] (**1**) in situ and used it as a precursor to form a six-coordinate model, [(2-CH_2CO-6-$MeOC_5H_3N$)Fe(CO)$_2$(2-S-6-MeC_5H_3N)] (**2**).[24] The sample of **1** prepared in situ was impure, and its reactions with monodentate thiolate ligands led to unidentified mixtures. We found that if the mixture prepared in situ and containing **1** was passed through an Al_2O_3 column immediately after its formation under an inert atmosphere, the crystalline and pure form of **1** could be isolated. The structure of **1** was determined by X-ray crystallography (Figure 1). The Fe center is in an octahedral coordination environment. It forms a five-membered metallacycle with the acylmethylpyridinyl moiety. The three CO ligands coordinate in a *fac* fashion. The acyl ligand executes a strong *trans* influence. As a result, the Fe–C_{CO} bond for the CO ligand *trans* to the acyl ligand is about 0.1 Å longer than that for the *cis* CO ligand. The IR data for the Fe–CO bonds are listed in Table 1, and they confirm the presence of three CO ligands.

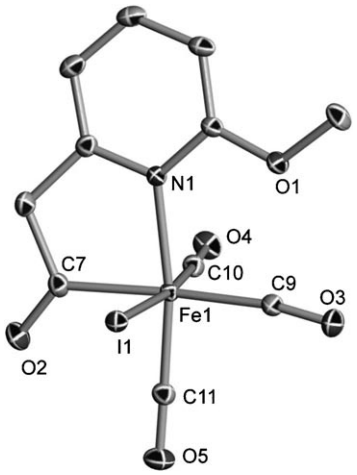


Figure 1. Solid-state structure of **1**. The thermal ellipsoids are displayed at 50% probability. Selected bond lengths [Å] and angles [°]: Fe1–N1 2.0439(9), Fe1–C7 1.9821(11), Fe1–C9 1.8994(11), Fe1–C10 1.7987(11), Fe1–C11 1.7921(11), Fe1–I1 2.6572(2), C9–O3 1.1355(14), C10–O4 1.1351(14), C11–O5 1.1432(14), C7–O2 1.2132(14); C7-Fe1-N1 83.51(4), C10-Fe1-I1 172.78(4), C11-Fe1-N1 170.76(5).

Once purified, **1** can react cleanly with monodentate thiolate ligands. Reaction of **1** with NaS(2,6-$Me_2C_6H_3$) produced a five-coordinate Fe complex [(2-CH_2CO-6-$MeOC_5H_3N$)Fe(CO)$_2${S-(2,6-$Me_2C_6H_3$)}] (**3**), accompanied by the loss of one CO molecule (Scheme 2). Complex **3** is diamagnetic. The IR spectrum shows two intense ν_{CO} absorption bands, consistent with the presence of two *cis*-orientated terminal CO ligands. The stretching frequencies are within several wavenumbers of those of [Fe]-hydrogenase (Table 1), thus indicating electronically very similar Fe

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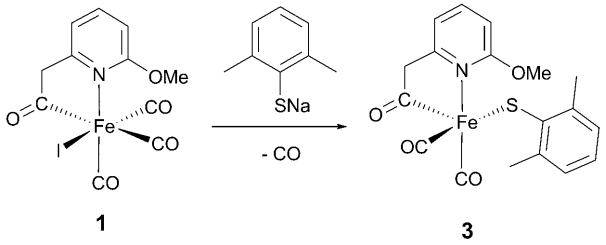
Table 1: Selected infrared data.

Complex	ν_{CO} [cm^{-1}]
1[a]	2084, 2018, 2003
2[a,b]	2026, 1961
3[a]	2013, 1950
3[c]	2022, 1959
5[c]	2073, 2024, 1995
[Fe]-hydrogenase[d]	2011, 1944
CO-inhibited [Fe]-hydrogenase[d]	2074, 2020, 1981
cofactor[d]	2031, 1972

[a] Spectrum of a solid sample. [b] Data from Ref. [24]. [c] Spectrum of a sample dissolved in THF. [d] Spectra of samples dissolved in water; data from Ref. [9].



1 3

Scheme 2. Synthesis of five-coordinate model complex **3** from isolated complex **1**.

centers. Compound **3** is not stable in solution at room temperature. In the dark, the half-life is about 5 h. Ambient light accelerates the decomposition of **3**, and the half-life is less than one hour. For this reason, the reactions of **3** were studied in the dark.

The solid-state molecular structure of **3** was established by X-ray crystallography (Figure 2). The coordination geometry of the Fe ion is best described as distorted square-pyramidal. The two CO ligands and the acyl donor are thus reflecting their strong *trans* influence. The sulfur ligand is *cis* to the acyl donor, and the position *trans* to the latter is vacant. The C2-Fe1-S1 angle of 162.50(18)° is closer to the 180° required for an ideal square-pyramidal geometry than to the 120° required for an ideal trigonal-bipyramidal geometry.

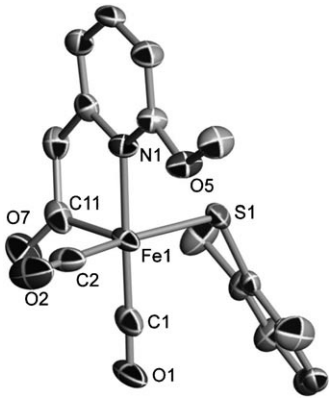
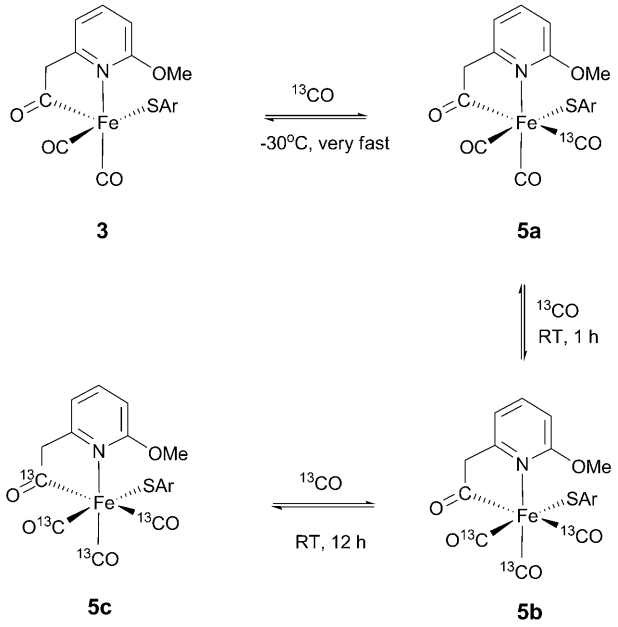


Figure 2. Solid-state structure of **3**. There are two independent molecules in each asymmetric unit; only one of them is shown. The thermal ellipsoids are displayed at 50% probability. Selected bond lengths [Å] and angles [°]: Fe1–N1 2.002(3), Fe1–S1 2.2163(12), Fe1–C11 1.915(5), Fe1–C1 1.773(5), Fe1–C2 1.786(5), C1–O1 1.155(5), C2–O2 1.144(5), C11–O7 1.209(5); C11-Fe1-N1 85.26(17), C1-Fe1-N1 177.08(17), C2-Fe1-S1 162.50(18).

Complex **3** reacted rapidly with CO to give [(2-CH_2CO-6-$MeOC_5H_3N$)Fe$(CO)_3${S-(2,6-$Me_2C_6H_3$)}] (**5**). The IR spectrum of **5** shows three intense ν_{CO} absorption bands, with frequencies similar to CO-inhibited [Fe]-hydrogenase[9] (Table 1). Complex **5** has a limited stability under CO and partially decomposes after one hour in THF at room temperature. However, **5** is stable at −30 °C for at least one week. The third CO ligand in **5** is labile, and purging a solution of **5** with N_2 or concentrating it in vacuo regenerates **3**.

The reaction of **3** with ^{13}CO in [D_8]THF was monitored by ^{13}C NMR spectroscopy.[26] The results could be rationalized by the reaction sequences described in Scheme 3. When **3** was exposed to ^{13}CO at −30 °C, complex **5a**, in which a ^{13}CO ligand occupies the originally vacant coordination site, was formed instantaneously. No further reaction occurred at −30 °C. However, exchange of unlabeled carbonyl and acyl groups with ^{13}CO was detected at room temperature. The exchange rates are different for the acyl and carbonyl carbon atoms. Within one hour, the two intrinsic CO ligands displayed ^{13}C labeling, giving rise to **5b**. After 12 h, the acyl carbon atom was partially labeled with ^{13}C to give **5c**.



3 5a 5c 5b

Scheme 3. Sequential reactions of **3** with ^{13}CO. Ar = 2,6-dimethylphenyl.

The isotopic exchange of the internal carbonyl group with ^{13}CO in **3** likely occurred via a five-coordinate intermediate that is a geometric isomer of **3**. The exchange of the acyl carbon atom could proceed first by elimination of carbonyl from the acyl ligand to form an iron alkyl species, which then reacts with ^{13}CO to incorporate the ^{13}C atom. For complex **3**, the rates for CO binding, exchange, and acyl exchange are significantly different, and CO binding is the fastest. It had

been shown that the internal CO ligands in [Fe]-hydrogenase did not undergo exchange with extrinsic CO within a short period of time.[9] The reactivity of **3** with ^{13}CO suggests that a longer time might be needed for exchange to be observed in [Fe]-hydrogenase.

The thiolate ligand in **3** was replaceable. Reaction of **3** with 2-mercapto-6-methylpyridine yielded complex **2**.[26] Complex **3** reacted rapidly with $[NEt_4]CN$ and [PPN]CN (PPN = bis(triphenylphosphine)iminium) at −30 °C, but no clean product could be isolated. Complex **3** also reacted with PPh_3 at room temperature to form a mixture of unidentified species. The initial products of these reactions are probably the six-coordinate species with CN^- or PPh_3 ligands, which happen to be highly unstable.

The five-coordinate complex **3**, however, did not react with simple donor ligands such as H_2O, CH_3CN, pyridine, and Et_3N. Like [Fe]-hydrogenase,[4] **3** did not react with O_2. The methoxy substituent on the pyridinyl ring might block the access of small molecules, yet **3** reacts with PPh_3 and 2-mercapto-6-methylpyridine, which are bigger than some of the unreactive donor ligands. Therefore, the lack of reactivity of **3** towards simple donors has an electronic origin. We suspect that two factors might be important: 1) the strong *trans* influence of the acyl ligand diminishes the ligand-binding affinity at the vacant site; 2) the Fe center in **3** is sufficiently electron-rich (as seen by the IR data of the complex) that it binds neither pure σ donors nor weak π acceptors, but only strong π acceptors.

The preparation of complex **3** sets the stage for biomimetic H_2 activation. A preliminary study showed that **3** did not mediate H/D exchange in a mixture of H^+, H_2, and D_2.[26] As activation of H_2 by [Fe]-hydrogenase only occurs in the presence of methenyl-H_4MPT^+,[27] we are currently developing small-molecule mimics of this unstable enzymatic substrate[28,29] for use in H_2 activation.

In summary, we describe the synthesis, structure, and reactivity of the first five-coordinate, square-pyramidal Fe^{II} model complex that reproduces the main structural features of the active site of [Fe]-hydrogenase. The structure of this complex and its lack of reactivity towards donor ligands suggest that the resting state of the Fe ion in [Fe]-hydrogenase could be five-coordinate.

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