Enzyme Models

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[Fe]-Hydrogenase Models Featuring Acylmethylpyridinyl Ligands**

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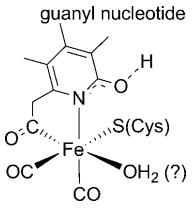
[Fe]-hydrogenase is a recently discovered hydrogenase that contains one single iron at each active site and no iron–sulfur cluster.[1,2] Also named as H_2-forming methylenetetrahydromethanopterin dehydrogenase (Hmd), this hydrogenase catalyzes the reversible reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT$^+$) with H_2 to form methylenetetrahydromethanopterin (methylene-H_4MPT) and H^+, an intermediary step in the reduction of CO_2 to methane by methanogens grown under nickel-limiting conditions.[3]

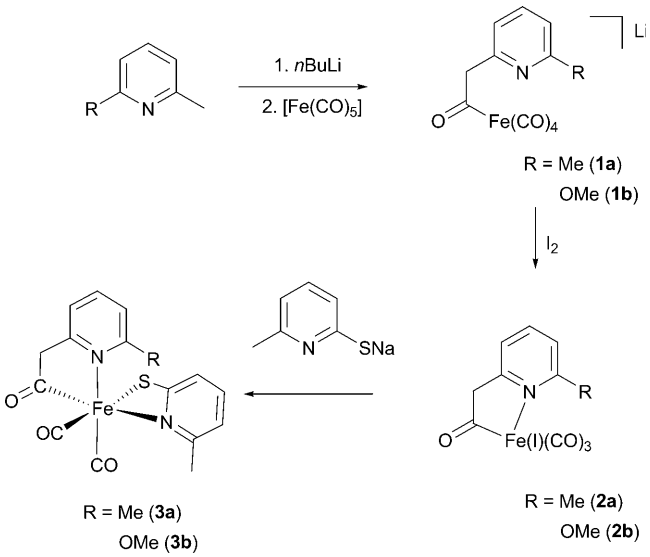
A multitude of spectroscopic and crystallographic tools have been employed to elucidate the structure of [Fe]-hydrogenase.[4–13] A unique iron-containing active site is found: the iron center is coordinated to a cysteine sulfur atom, two *cis*-CO ligands, a bidentate pyridone cofactor, and a yet unknown ligand, which is probably a disordered solvent molecule (Scheme 1).[10–12] The structure of the pyridone cofactor is intriguing, as it binds to iron through a acylmethylpyridinyl moiety.[11] The same cofactor has not been found in other enzymes.



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

The pyridone cofactor most likely bears important functions. The acyl ligand is *trans* to the vacant coordination site where H_2 presumably binds. Whereas the mechanism of H_2 activation by [Fe] hydrogenase is still unclear, especially because prior methenyl-H_4MPT$^+$ binding to the enzyme is a prerequisite,[12] the strongly *trans*-influencing acyl ligand[14] might be one of nature's design elements for this chemistry. The pyridinyl part of the cofactor might also be essential in setting the correct coordination sphere at the iron center. Although a number of synthetic mimics of [Fe] hydrogenase have been reported recently,[15–21] none of them contains the bidentate acylmethylpyridinyl unit. Herein we describe the synthesis, structure, and reactivity of such model complexes.

The acylmethylpyridinyl unit was assembled on the iron center by treatment of the in-situ generated methylpyridinyl anion with $Fe(CO)_5$, similar to the Fischer route to metal acyl species[22] (Scheme 2). The resulting complexes have formulas Li[(6-R-C_4H_3N-2-CH_2CO)Fe(CO)$_4$] (R = Me (**1a**), OMe (**1b**)). Judging from the IR spectra, which show three ν(CO) bands, the pyridinyl nitrogen atom is not coordinating



Scheme 2. Synthesis of model iron complexes. For details of the reaction conditions, see the Supporting Information.

and the geometry of the iron center is trigonal-bipyramidal in **1a** and **1b**. Reactions of **1a** and **1b** with I_2 gave presumably [(6-R-C_4H_3N-2-CH_2CO)Fe(CO)$_3$I] (**2a** and **2b**), which are too unstable to be isolated. Further reactions of **2a** and **2b** with sodium 6-methyl-2-mercaptopyridinate, on the other hand, produced the desired complexes [(6-R-C_4H_3N-2-CH_2CO)Fe(CO)$_2$(6-Me-C_4H_3N-2-S)] (**3a** and **3b**, Scheme 2) that were isolated and purified.[23]

In the IR spectra of **3a** and **3b**, two intense ν(CO) absorption bands were observed, revealing the presence of two *cis*-orientated terminal carbonyl ligands.[23] The ν(max) values are nearly identical (2029 and 1962 cm^{-1} for **3a**, and 2026 and 1961 cm^{-1} for **3b**). The Fe–CO stretching frequencies fall within the range for iron(II) bis(carbonyl) complexes.[15] The iron centers in **3a** and **3b** are slightly less electron-rich than in [Fe]-hydrogenase ($\tilde{\nu}$(max) = 2011 and 1944 cm^{-1}).[4]

The molecular structure of **3a** was confirmed by X-ray crystallography (Figure 1). The active site of [Fe] hydrogenase is mimicked well. The iron center is six-coordinate, and the acylmethylpyridinyl moiety forms a five-membered metallacycle with the iron center. The Fe–C(acyl) bond length is comparable to those found in other iron(II) acyl compounds.[17,19,24] The two CO ligands occupy the positions

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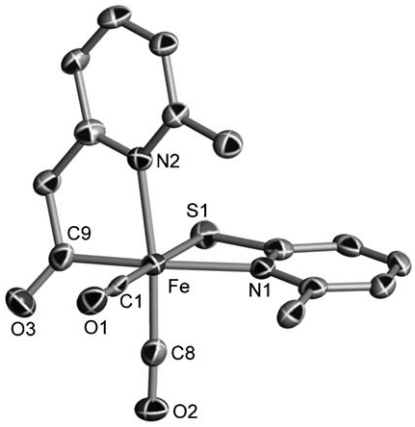


Figure 1. Solid-state structure of **3a**. Thermal ellipsoids are set at 50% probability. Selected bond distances [Å] and angles [°]: Fe–N1 2.111(5), Fe–S1, 2.376(2), Fe–N2 2.082(5), Fe–C9 1.946(6), Fe–C1 1.776(6), Fe–C8 1.778(6), C1–O1 1.144(7), C8–O2 1.149(7), C9–O3 1.210(7); C8-Fe-C1 91.3(3), N2-Fe-C9 83.3(2), N1-Fe-S1 69.61(15).

cis to the acyl ligand, probably as a consequence of the strong *trans* influence of both carbonyl and acyl ligands. Although the sulfur ligand has a choice of two sites, it chooses to occupy the position *trans* to carbonyl rather than *trans* to acyl. We[19] and others[17] have found that in diiron(II) dithiolate acyl complexes, the Fe–S bonds are longer when the thiolate ligands are *trans* to acyl than when they are *trans* to carbonyl ligands. Taken together, these observations suggest that for an iron(II) ion, an acyl ligand has a stronger *trans* influence than a carbonyl ligand. Therefore, the stereochemistry of the coordination sphere of the iron center in [Fe]-hydrogenase is probably dictated by the electronic properties of the ligands. The reproduction of the same stereochemistry in model complexes **3a** and **3b** probably has the same electronic origin.

Complexes **3a** and **3b** undergo CO exchange under one atmosphere of CO gas.[23] The reactions could be probed using ^{13}C NMR spectroscopy. Figure 2a shows the ^{13}C NMR spectrum of **3b**. The signals at $\delta = 211.9$ and 214.6 ppm are attributed to the carbonyl carbons, and the signal at $\delta =$ 261.7 ppm originates from the acyl carbon atom. Their intensities are low compared to those of the carbon atoms on the pyridinyl ring ($\delta = 100$–150 ppm). Upon addition of ^{13}CO, all signals remain at the same positions. However, the intensities of the signals from the acyl and carbonyl carbon atoms increase gradually and significantly (Figure 2b). The change in the ^{13}C NMR spectra reflects the incorporation of ^{13}C in the acyl and carbonyl ligands (Figure 2c). The exchange reaches completion over a week (in dark or under white visible light).[23] Following the time course of the reaction showed that the incorporation of three ^{13}C atoms occurred at a similar rate. The IR data further confirm the ^{13}CO exchange: the ν(CO) are at 1968 and 1904 cm^{-1} for ^{13}CO-exchanged **3b^{C13}**.

We have reported the rapid CO exchange reaction of a diiron(II) dithiolate complex that proceeds through a monomeric iron tris(carbonyl) intermediate.[19] The observation of similar, albeit slower, CO exchange with the six-coordinate mononuclear complex **3b** is interesting. A different mechanism is operating here; we propose that a key step is the elimination of carbonyl from the acyl ligand to form a iron alkyl species (**4b**). This step might be preceded by an initial carbonyl dissociation to generate a five-coordinate intermediate. Complex **4b** then reacts with ^{13}CO to incorporate the first ^{13}C element (Figure 2c). Repeating the same process two more times would lead to the final product **3b^{C13}**.

The CO exchange reaction was accelerated by photolysis (UV-A light),[23] again suggesting a possible CO dissociation step. However, an isomerization reaction also took place under the same conditions. Thus, a new complex was clearly

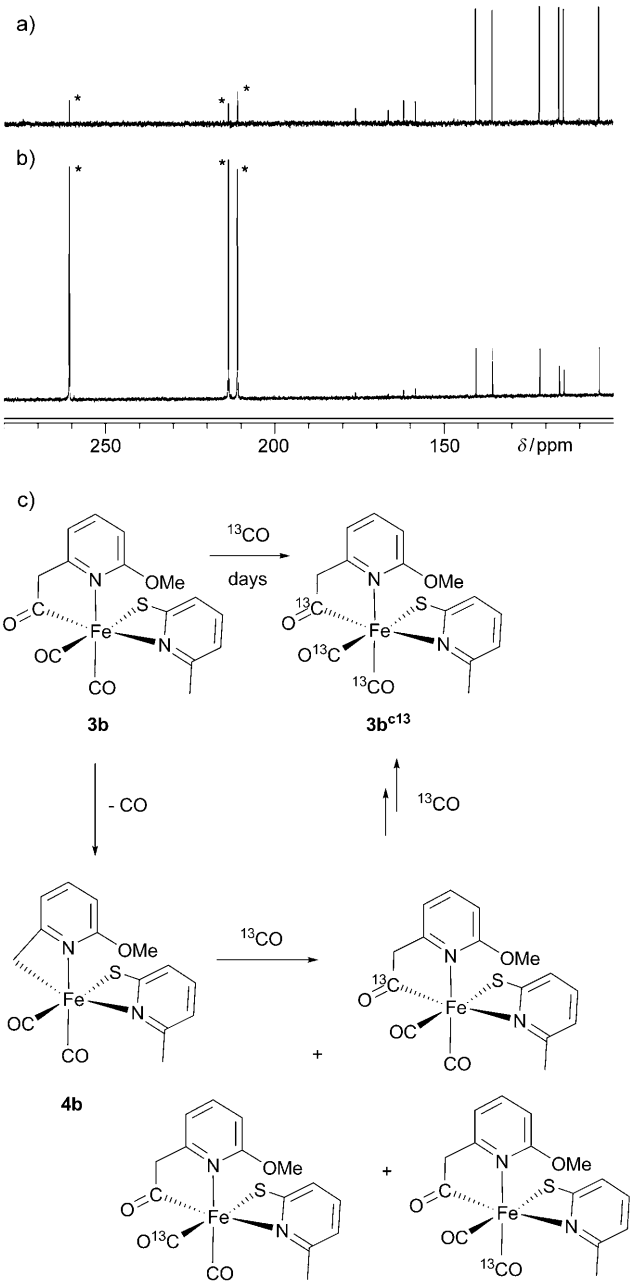
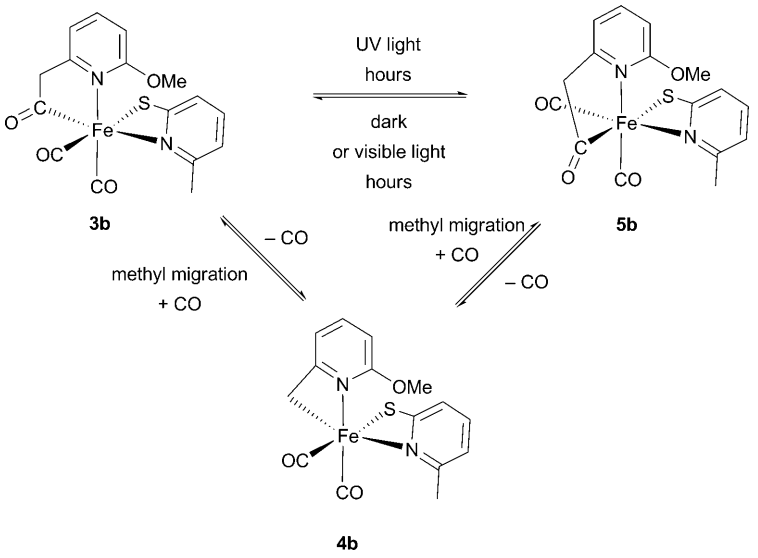


Figure 2. CO exchange reactivity of **3b**. a) ^{13}C NMR spectrum of **3b**; b) ^{13}C NMR spectrum of **3b** + ^{13}CO; the asterisks designate the signals from acyl and carbonyl carbon atoms. c) A possible mechanism for the CO exchange reaction.



Scheme 3. Isomerization of complex **3b** under UV-A light.

observed after 2 h in the solution of **3b** or **3b**^{13C} under UV-A light (365 nm)[23] and in the absence of extrinsic CO. The NMR spectra of this species are consistent with an iron complex having the same formula as **3b**. This species is therefore assigned as the isomer **5b** in which the acyl ligand is now *trans* to the sulfur ligand. A possible mechanism for the formation of this complex is depicted in Scheme 3. The reaction occurred via the same intermediate **4b**, the generation of which was faster under UV light. Methyl migration and subsequent carbonyl binding could lead to two isomers, **3b** and **5b**, both of which were observed. As discussed earlier, **5b** is less stable due to the stronger *trans* influence of the acyl ligand. Indeed, in the absence of UV light, **5b** isomerized to form **3b** (Scheme 3).

In summary, we have described the synthesis and reactivity of model complexes of the [Fe]-hydrogenase containing an intriguing acylmethylpyridinyl moiety. The structure of the model complexes suggests a strong *trans* influence of the acyl ligand, a factor to consider in understanding the mechanism of H_2 activation by [Fe]-hydrogenase. The CO exchange and isomerization reactivity show that iron acyl carbonyl complexes are subject to dynamic equilibriums involving insertion, migration, and elimination reactions. These reactions could be relevant to the chemistry of [Fe]-hydrogenase. For example, it had been shown that the extrinsic CO ligand did not exchange with the internal CO ligands in [Fe]-hydrogenase.[4] However, the measurement was carried out in the absence of UV-A light and shortly under the introduction of ^{13}CO. We now find that for a six-coordinate complex such as **3b**, the exchange takes hours to be observed. Re-examination of the data from the enzymatic study might be worthwhile. Even though complexes **3a** and **3b** did not react with H_2, their reactivity suggests that H_2 activation under photolysis conditions using similar models might be feasible.

Experimental Section

3a: *n*BuLi (1.0 mL, 1.6 M, 1.6 mmol) was added into a solution of 2,6-dimethylpyridine (171.2 mg, 1.6 mmol) in THF (10 mL) under stirring at 0 °C. After stirring for 0.5 h at this temperature, the solution was added into a solution of $[Fe(CO)_5]$ (313.6 mg, 1.6 mmol) in THF (15 mL) at −50 °C. The resulting solution was warmed slowly to −20 °C (about 2 h), followed by I_2 (406.4 mg, 1.6 mmol) at −60 °C. The resulting solution was kept at −60 °C for 2 h, followed by adding a solution of sodium 6-methyl-2-pyridinethiolate (235.2 mg, 1.6 mmol) in THF (10 mL) at −60 °C. The solution was kept at this temperature overnight. The solvent was then evaporated in vacuum, and the residue was extracted with CH_2Cl_2 (10 mL). After removing the solvent of the filtrate, the residue was placed in an Al_2O_3 column. Elution with 10:1 CH_2Cl_2/acetone (v/v) developed a yellow band, which afforded **3a** (150.0 mg, 0.41 mmol, 25 %) as yellow crystals after crystallization.

^1H NMR (400 MHz, CD_3CN): $\delta = 7.71$ (t, $J = 7.2$ Hz, 1H), 7.32 (m, 2H), 7.17 (d, $J = 7.2$ Hz, 1H), 6.80 (d, $J = 8.0$ Hz, 1H), 6.54 (d, $J = 7.2$ Hz, 1H), 4.47 (d, $J = 20.0$ Hz, 1H), 3.82 (d, $J = 20.0$ Hz, 1H), 2.52 (s, 3H), 2.20 ppm (s, 3H). ^{13}C NMR (100 MHz, $[D_6]$DMSO): $\delta = 265.2$ (CH_2CO), 213.3 (terminal *C*O), 210.0 (terminal *C*O), 175.1, 162.1, 160.0, 158.1, 138.4, 137.5, 124.7, 122.2, 119.9, 117.9, 65.0, 24.7, 22.3 ppm. IR: $\tilde{\nu} = 2029$ (s, terminal CO), 1962 cm^{-1} (s, terminal CO). Elemental analysis (%) calcd for $C_{16}H_{14}FeN_2O_3S{\cdot}0.1\,CH_2Cl_2$: C 51.1, H 3.8, N 7.4; found: C 50.9, H 3.9, N 7.3.

A Fischer Bioblock Scientific UV lamp (VL-6.LC; 365 nm and 254 nm, 2 × 6 W) was used for the reactions under UV-A light: 365 nm, intensity 350/390 μW cm^{-2}.

For further experimental, characterization, and crystallographic details, see the Supporting Information.

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