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A Pyridinol Acyl Cofactor in the Active Site of [Fe]-hydrogenase Evidenced by the Reactivity of Model Complexes

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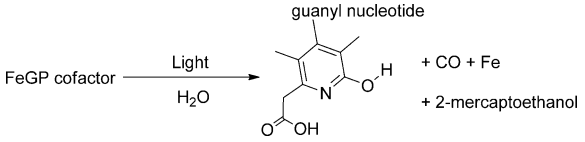
[Fe]-hydrogenase, containing an iron guanylylpyridinol (FeGP) cofactor, can split H_2 into H^+ and H^- in the presence of methenyltetrahydromethanopterin (methenyl-H_4MPT^+).[1–3] The FeGP cofactor can be released by denaturation of the enzyme in the presence of 2-mercaptoethanol.[4–6] The protein-free cofactor is too unstable to be identified. When irradiated by UV-A light, the extracted cofactor decomposed to iron, CO, 2-mercaptoethanol, and an organic pyridinol carboxylic acid derivative (Scheme 1).[6,7]



Scheme 1. Decomposition of the extracted FeGP cofactor under UV-A light.

This observation was the basis of an earlier structural model in which the pyridinol moiety coordinated to the Fe ion only through its N atom.[8]

This structural model was later revised based on the X-ray structure of a mutated [Fe]-hydrogenase.[9] Currently, it is thought that the Fe^{II} center in the active site of [Fe]-hydrogenase is coordinated with two *cis*-CO, a cysteine sulfur atom (Cys 176), and the bidentate pyridinol acyl ligand through its nitrogen atom and acyl donors (Figure 1 **A**).[9–11] The structure of the extracted FeGP cofactor was proposed accordingly (Figure 1 **B**).[7,12]

Recent infrared spectroscopic and mass spectrometric studies of [Fe]-hydrogenase provide evidence for the acyl-iron ligation.[11,12] As no other protein containing an acyl-iron ligation has been found in the biological systems, more

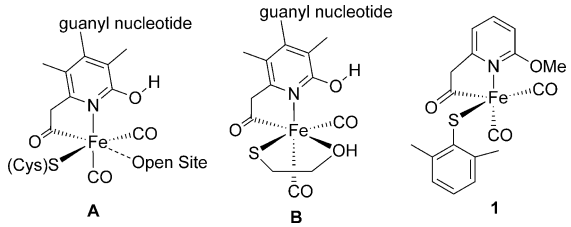
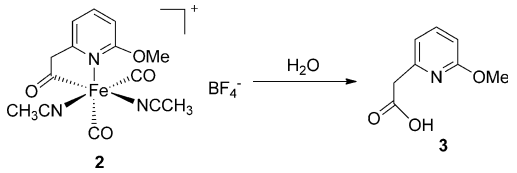


Figure 1. Proposed structure for the active site of [Fe]-hydrogenase (**A**). Proposed structure for the protein-free FeGP cofactor extracted with 2-mercaptoethanol (**B**). Active site model (**1**).[27,28]

experimental evidence for the existence of this unique moiety in [Fe]-hydrogenase is warranted.[12]

Based on the proposed structure of the active site of [Fe]-hydrogenase, a number of small molecular mimics have been prepared.[13–28] Recently we reported the synthesis and protonation reactions of a 5-coordinate model [(2-CH_2CO-6-$MeOC_5H_3N$)$Fe(CO)_2${S-(2,6-$Me_2C_6H_3$)}] (**1**, Figure 1).[27,28] The reaction with HBF_4·Et_2O afforded an ionic complex [(2-CH_2CO-6-$MeOC_5H_3N$)$Fe(CO)_2(CH_3CN)_2$](BF_4) (**2**).[28] We found that **2** is somewhat soluble in water, which prompted us to explore its reactivity with water. If the reaction could occur similar to that described in Scheme 1, the acyl-iron group in [Fe]-hydrogenase would be further proved. Furthermore, to confirm the detailed geometry of the protein-free FeGP cofactor extracted with 2-mercaptoethanol, and which group Cu^+ attacks when reacts with [Fe]-hydrogenase, here we also describe the reactivity of **1** with 2-mercaptoethanol and the Cu^+ ion.

Complex **2** reacts with water to form an isolable organic product that was identified as **3** (Scheme 2). The ^1H NMR spectrum of **3** exhibits three signals at $\delta = 7.65$, 6.83, and 6.76 ppm for the pyridine ring, one singlet at $\delta = 3.98$ ppm for the methoxy group, and one singlet at $\delta = 3.83$ ppm for the methylene group. In the ^{13}C NMR spectrum of **3**, one



Scheme 2. Reaction of **2** with water.

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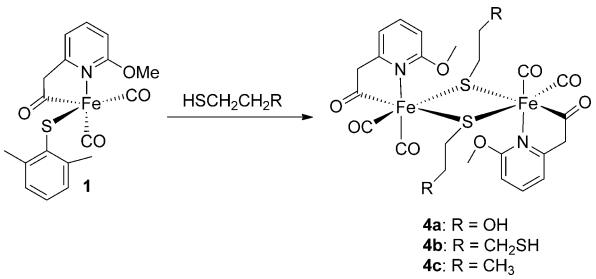
signal of carboxyl group ($\delta = 171.3$ ppm), five signals for the pyridine ring ($\delta = 163.0$, 151.1, 140.0, 116.3, and 109.8 ppm), one signal for the methoxy group ($\delta = 53.9$ ppm), and one signal for the methylene group ($\delta = 40.5$ ppm) are observed. Based on the NMR data, compound **3** was assigned as [(2-CH_2COOH-6-$MeOC_5H_3N$)], which was further confirmed by high-resolution mass spectrometry (HRMS).[29] The structure of **3** is quite similar to the organic product isolated from the reaction of FeGP cofactor with water (Scheme 1), which provides evidence for the existence of the iron-acyl ligation, and thus, the pyridinol acyl cofactor in the active site of [Fe]-hydrogenase.

Although the composition of the protein-free FeGP cofactor is determined, its structure is not established.[7] To probe the structure of the FeGP cofactor extracted by 2-mercaptoethanol, we then attempted to prepare model complexes with a 2-mercaptoethanol ligand. Reaction of [(2-CH_2CO-6-$MeOC_5H_3N$)$Fe(CO)_3I$][27] with $NaSCH_2CH_2OH$ was investigated. However, only unidentified mixtures were produced.

The protein-free FeGP cofactor was isolated by the transthiolation of [Fe]-hydrogenase.[4–6] Gratifyingly, the target model complex could be prepared in a similar manner. Thus, reaction of **1** with $SHCH_2CH_2OH$ (2 equiv) at −20 °C gave [(6-MeO-C_5H_3N-2-CH_2CO)$Fe(CO)_2(SCH_2CH_2OH)]_2$ (**4a**) (Scheme 3). Compound **4a** is unstable at room temperature, and its half-life is about 0.5 h. The ^1H NMR spectrum of **4a** exhibits three signals at $\delta = 7.84$, 7.11, and 6.51 ppm for the pyridine rings, two doublets at $\delta = 4.73$ and 3.53 ppm for the diastereotopic methylene hydrogens, one singlet at $\delta = 3.39$ ppm for the methoxy groups, and four multiplets between $\delta = 3.70$–2.45 ppm for -CH_2CH_2OH groups.[29] The IR spectra of **4a** show four intense ν(CO) absorption bands both in the solid state and in solution (Table 1).[29] The ^1H NMR and IR data indicates a dimeric structure for **4a**. Four instead of three signals were observed for the -CH_2CH_2OH groups in the ^1H NMR spectrum. This is probably caused by the bulky environment around the Fe centers, which restricts the rotation of the S–CH_2 bonds. A similar phenomenon was observed in complex [(6-MeO-C_5H_3N-2-CH_2CO)$Fe(CO)_3${S-(2,6-$Me_2C_6H_3$)}] (**5**), which was formed from the reaction of **1** with CO.[27] At −30 °C, the two methyl groups in the thiophenol ligand in **5** show two singlets in the ^1H NMR spectrum due to the restricted rotation around the S-C_6H_3 bond.[27]



Scheme 3. Transthiolation reactions of **1**.

Table 1. Selected infrared data.

Complex	ν(CO) [cm^{-1}]
4a[a]	2023, 1999, 1978, 1953
4a[b]	2021, 2003, 1961, 1946
4b[a]	2017, 1997, 1939
4b[b]	2018, 2000, 1955, 1943
4c[a]	2017, 1992, 1943, 1930
4c[b]	2019, 2001, 1958, 1944
FeGP cofactor[c]	2004, 1934
FeGP cofactor[d]	2031, 1972

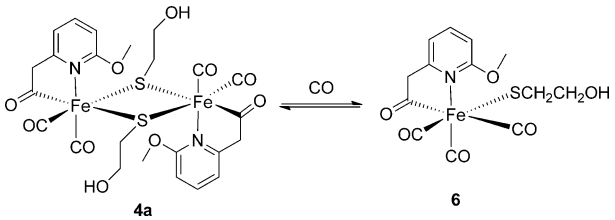
[a] Spectrum of a solid sample. [b] Spectrum of a sample dissolved in CH_3CN. [c] Spectrum of a solid sample; data taken from ref. [12]. [d] Spectrum of a sample dissolved in water; data taken from ref. [30].

Reaction of **1** with $HSCH_2CH_2CH_2SH$ gave a similar product [(6-MeO-C_5H_3N-2-CH_2CO)$Fe(CO)_2(SCH_2CH_2CH_2SH)]_2$ (**4b**) (Scheme 3). Compound **4b** is highly unstable; it decomposes even at −30 °C in solution.

Treatment of **1** with $HSCH_2CH_2CH_3$ gave [(6-MeO-C_5H_3N-2-CH_2CO)$Fe(CO)_2(SCH_2CH_2CH_3)]_2$ (**4c**) (Scheme 3). The ^1H NMR and IR spectra of **4c** are similar to those of **4a** and **4b**, which indicates that the -OH and -SH groups in **4a** and **4b** do not coordinate to the Fe centers.

The ligands of the iron centers in **4a** and in the protein-free FeGP cofactor are similar. However, the extracted FeGP cofactor is a monomeric iron complex according to the IR data (Table 1). The reason for the structural discrepancy is still unknown, but is probably caused by the difference of the second coordination sphere.[31]

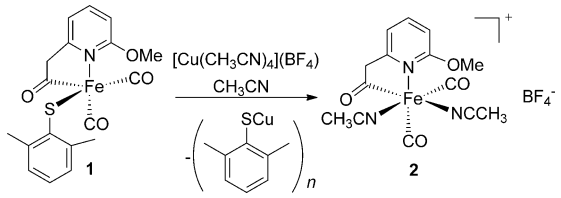
The protein-free FeGP cofactor was shown to bind an external CO ligand.[32] Similarly, compound **4a** reacted with CO reversibly to give a tricarbonyl product [(6-MeO-C_5H_3N-2-CH_2CO)$Fe(CO)_3(SCH_2CH_2OH)$] (**6**, Scheme 4).



Scheme 4. Reversible reaction of **4a** with CO.

This compound might be considered as a mimic for the extra-CO-bound extracted cofactor.

[Fe]-hydrogenase is sensitive to Cu^+ ions, however, the origin of the inhibition is unknown;[33] therefore, the reaction of **1** with $[Cu(CH_3CN)_4](BF_4)$ was studied (Scheme 5). The reaction yielded complex **2**[28] and an insoluble salt with the composition of $[Cu\{S\text{-}(2,6\text{-}Me_2C_6H_3)\}]_n$.[34] Thus, the Cu^+ ion attacked the thiolate ligand in **1**. This result suggests that the inhibition of [Fe]-hydrogenase by Cu^+ ion might be due to the attack of Cu^+ on the Cys176 ligand.



Scheme 5. Reaction of **1** with Cu^+.

In summary, the reaction of **2** with water is a good chemical model for the decomposition of the protein-free FeGP cofactor. Both reactions produce pyridinyl carboxylic acid derivatives. This result gives strong evidence for the existence of the unique pyridinol acyl cofactor in the active site of [Fe]-hydrogenase. It is the first time that evidence has been provided by the reactivity of a well-defined model complex. Furthermore, to model the protein-free FeGP cofactor, 2-mercaptoethanol was introduced successfully to Fe center, although this results in a dimer **4a** instead of a monomer, which is different from the FeGP cofactor. Additionally, the reactivity of complex **1** with Cu^+ suggests that Cu^+ inhibits [Fe]-hydrogenase by attacking the Cys176 ligand.

Acknowledgements

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Keywords: enzyme models · cofactors · hydrogenase · iron · water splitting

[1] R. K. Thauer, A. R. Klein, G. C. Hartmann, *Chem. Rev.* **1996**, *96*, 3031–3042.
[2] S. Shima, R. K. Thauer, *Chem. Rec.* **2007**, *7*, 37–46.
[3] R. K. Thauer, A. K. Kaster, M. Goenrich, M. Schick, T. Hiromoto, S. Shima, in *Annual Review of Biochemistry Vol. 79*, **2010**, pp. 507–536.
[4] G. Buurman, S. Shima, R. K. Thauer, *FEBS Lett.* **2000**, *485*, 200–204.
[5] E. J. Lyon, S. Shima, G. Buurman, S. Chowdhuri, A. Batschauer, K. Steinbach, R. K. Thauer, *Eur. J. Biochem.* **2003**, *271*, 195–204.
[6] S. Shima, E. J. Lyon, M. S. Sordel-Klippert, M. Kauss, J. Kahnt, R. K. Thauer, K. Steinbach, X. L. Xie, L. Verdier, C. Griesinger, *Angew. Chem.* **2004**, *116*, 2601–2605; *Angew. Chem. Int. Ed.* **2004**, *43*, 2547–2551.
[7] S. Shima, U. Ermler, *Eur. J. Inorg. Chem.* **2011**, 963–972.
[8] S. Shima, O. Pilak, S. Vogt, M. Schick, M. S. Stagni, W. Meyer-Klaucke, E. Warkentin, R. K. Thauer, U. Ermler, *Science* **2008**, *321*, 572–575.
[9] T. Hiromoto, K. Ataka, O. Pilak, S. Vogt, M. S. Stagni, W. Meyer-Klaucke, E. Warkentin, R. K. Thauer, S. Shima, U. Ermler, *FEBS Lett.* **2009**, *583*, 585–590.
[10] T. Hiromoto, E. Warkentin, J. Moll, U. Ermler, S. Shima, *Angew. Chem.* **2009**, *121*, 6579–6582; *Angew. Chem. Int. Ed.* **2009**, *48*, 6457–6460; *Angew. Chem.* **2009**, *121*, 6579–6582.
[11] M. Schick, X. Xie, K. Ataka, J. Kahnt, U. Linne, S. Shima, *J. Am. Chem. Soc.* **2012**, *134*, 3271–3280.
[12] S. Shima, M. Schick, J. Kahnt, K. Ataka, K. Steinbach, U. Linne, *Dalton Trans.* **2012**, *41*, 767–771.
[13] C. Tard, C. J. Pickett, *Chem. Rev.* **2009**, *109*, 2245–2274.
[14] J. A. Wright, P. J. Turrell, C. J. Pickett, *Organometallics* **2010**, *29*, 6146–6156.
[15] M. J. Corr, J. A. Murphy, *Chem. Soc. Rev.* **2010**, *39*, 2279–2292.
[16] X. F. Wang, Z. M. Li, X. R. Zeng, Q. Y. Luo, D. J. Evans, C. J. Pickett, X. M. Liu, *Chem. Commun.* **2008**, 3555–3557.
[17] B. V. Obrist, D. F. Chen, A. Ahrens, V. Schunemann, R. Scopelliti, X. L. Hu, *Inorg. Chem.* **2009**, *48*, 3514–3516.
[18] A. M. Royer, T. B. Rauchfuss, D. L. Gray, *Organometallics* **2009**, *28*, 3618–3620.
[19] B. Li, T. Liu, C. V. Popescu, A. Bilko, M. Y. Darensbourg, *Inorg. Chem.* **2009**, *48*, 11283–11289.
[20] T. B. Liu, B. Li, C. V. Popescu, A. Bilko, L. M. Perez, M. B. Hall, M. Y. Darensbourg, *Chem. Eur. J.* **2010**, *16*, 3083–3089.
[21] D. F. Chen, R. Scopelliti, X. L. Hu, *J. Am. Chem. Soc.* **2010**, *132*, 928–929.
[22] D. F. Chen, R. Scopelliti, X. L. Hu, *Angew. Chem.* **2010**, *122*, 7674–7677; *Angew. Chem. Int. Ed.* **2010**, *49*, 7512–7515; *Angew. Chem.* **2010**, *122*, 7674–7677.
[23] P. J. Turrell, J. A. Wright, J. N. T. Peck, V. S. Oganesyan, C. J. Pickett, *Angew. Chem.* **2010**, *122*, 7670–7673; *Angew. Chem. Int. Ed.* **2010**, *49*, 7508–7511; *Angew. Chem.* **2010**, *122*, 7670–7673.
[24] A. M. Royer, M. Salomone-Stagni, T. B. Rauchfuss, W. Meyer-Klaucke, *J. Am. Chem. Soc.* **2010**, *132*, 16997–17003.
[25] S. Tanino, Y. Ohki, K. Tatsumi, *Chem. Asian J.* **2010**, *5*, 1962–1964.
[26] D. F. Chen, A. Ahrens-Botzong, V. Schuemann, R. Scopelliti, X. L. Hu, *Inorg. Chem.* **2011**, *50*, 5249–5257.
[27] D. F. Chen, R. Scopelliti, X. L. Hu, *Angew. Chem. Int. Ed.* **2011**, *50*, 5670–5672; *Angew. Chem.* **2011**, *123*, 5789–5791.
[28] D. F. Chen, R. Scopelliti, X. L. Hu, *Angew. Chem. Int. Ed.* **2012**, *51*, 1919–1921; *Angew. Chem.* **2012**, *124*, 1955–1957.
[29] See the Supporting Information for details.
[30] E. J. Lyon, S. Shima, R. Boecher, R. K. Thauer, F. W. Grevels, E. Bill, W. Roseboom, S. P. J. Albracht, *J. Am. Chem. Soc.* **2004**, *126*, 14239–14248.
[31] X. Yang, M. B. Hall, *J. Am. Chem. Soc.* **2009**, *131*, 10901–10908.
[32] S. Shima, E. J. Lyon, R. K. Thauer, B. Mienert, E. Bill, *J. Am. Chem. Soc.* **2005**, *127*, 10430–10435.
[33] S. Shima, R. K. Thauer, U. Ermler, *Met. Ions Life Sci.* **2009**, *6*, 219–240.
[34] M. D. Janssen, M. A. Corsten, A. L. Spek, D. M. Grove, G. van Koten, *Organometallics* **1996**, *15*, 2810–2820.

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Enzyme Models

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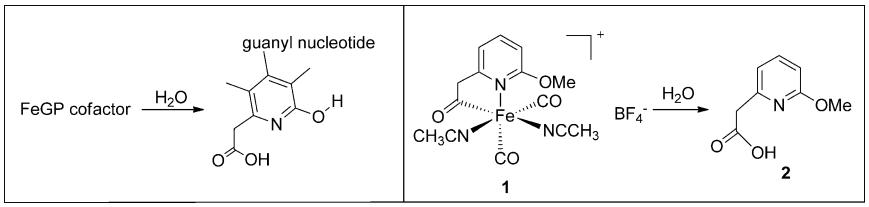
A Pyridinol Acyl Cofactor in the Active Site of [Fe]-hydrogenase Evidenced by the Reactivity of Model Complexes



Understanding the complex: The decomposition reaction of a water-soluble complex (see scheme; **1**) in H_2O confirms the existence of a unique bidentate pyridinol cofactor in [Fe]-hydrogenase. This unique moiety is confirmed for the first time by the decomposition of a well-defined model complex containing a pyridinyl methyl acyl ligand.