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Synthesis and Reactivity of Iron Acyl Complexes Modeling the Active Site of [Fe]-Hydrogenase

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[Fe]-hydrogenase, or H_2-forming methylene-tetrahydromethanopterin dehydrogenase (Hmd), is the third class of hydrogenase.[1] It catalyzes the reduction of methenyl-tetrahydromethanopterin (methenyl-H_4MPT^+) with H_2 to form methylene-tetrahydromethanopterin (methylene-H_4MPT) and H^+.[2] This is an intermediate step in the reduction of CO_2 to methane by some methanogens, and it occurs likely through heterolytic H_2 activation.[3] The structure of [Fe]-hydrogenase, and especially its active site, has been subjected to extensive spectroscopic and structural studies.[4−8] The current model suggests that the active site contains an iron center coordinated to a cysteine sulfur atom, two *cis*-CO ligands, a bidentate pyridone molecule through its nitrogen and acyl carbon atoms, and a yet unidentified ligand (Figure 1).[6,7] This active site is unique compared to those of [FeFe]- and [FeNi]-hydrogenases,[9] as it hosts a mono-Fe center and an unusual acyl-derivatized pyridone cofactor.

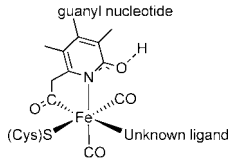

Figure 1. Structure of the active site of [Fe]-hydrogenase.

A few synthetic mimics of [Fe]-hydrogenase have been reported.[10−12] However, they model only a fraction of the structural features of the active site. Moreover, their reactivities are rarely reported. Here we describe the synthesis and reactivity of Fe acyl complexes, including those reproducing the first coordination sphere of the Fe ion in [Fe]-hydrogenase. The model complexes show interesting CO-exchange behavior.

Our synthetic strategy involved the installation of an acyl ligand in an early stage (Figure 2). Reaction of **2**[13] with sodium 6-methyl-2-mercaptopyridinate produced the diiron dithiolate complex **3** with concomitant salt eliminations.

The structure of **3** was confirmed by X-ray crystallography (Figure 2).[14] The complex is a [$Fe^{II}Fe^{II}$] homodimer bridged by the two sulfur donors of the pyridinyl thiolate ligand. The Fe−C distances for the CO and acyl ligands are comparable to those in analogous Fe^{II} complexes.[11,15] The [Fe_2S_2] core is asymmetric with a longer Fe−S distance of ∼2.47 Å and a shorter distance of ∼2.36 Å. Similar features were noted in a recently synthesized diiron(II) dithiolate complex.[11]

3 reacted rapidly with phosphine, isocyanide, and cyanide to form 6-coordinate and mononuclear Fe^{II} complexes **4**-**6** (Figure 2). These complexes were characterized by NMR, IR, Mass Spec, elemental analysis, and in the case of **6**, also X-ray crystallography.[14] The structure of **6** shows that the phosphine occupies a position *trans* to the acetyl ligand. Because complexes **4**−**6** are iso-electronic, the sixth ligand (CN^-, RNC) in **4** and **5** most likely occupies the same position in each.

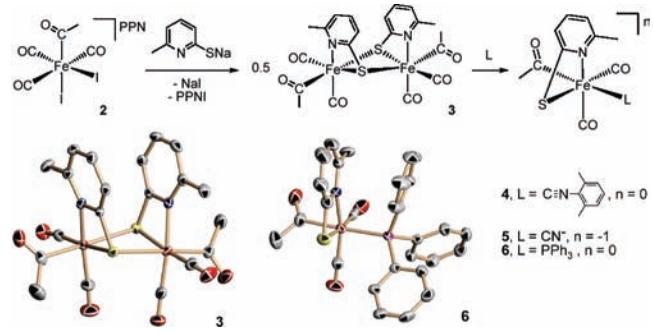

Figure 2. Synthesis of complexes **3**−**6**, and structures of **3** and **6**. The thermal ellipsoids are displayed in 50% probability. The counteranion for **5** is bis(triphenylphosphine)iminium (PPN).

Complexes **4**−**6** are structural mimics of [Fe]-hydrogenase. The Fe ions are coordinated by the same set of five donor atoms as in the enzyme. Moreover, the stereochemistry of the coordination polyhedron of Fe ion is completely reproduced. In the enzyme, an unidentified ligand occupies the position *trans* to the acyl ligand; in the synthetic complexes, CN^-, RNC, or PPh_3 occupies this position. It was reported that CN^- is a weak inhibitor of [Fe]-hydrogenase.[5] Complex **5** could then be considered as a mimic for the CN^--inhibited [Fe]-hydrogenase.

Selected IR data for **3**−**6** are listed in Table S1, SI.[14] The CO/CN stretching frequencies in **4**−**6** are comparable to those of the enzyme in various states, as well as some other monomeric Fe^{II} dicarbonyl species.[5,10,11]

[Fe]-hydrogenase binds reversibly an extrinsic CO. This reactivity is an indication of some Lewis acidity at the Fe center, which is a prerequisite for H_2 binding.[16] The reaction of **3** with CO was thus investigated. Upon exposure of **3** in $CDCl_3$ to 1 atm of CO, the solution immediately changed from orange to yellow. According to ^{1}H NMR, two species were formed in a ratio of ca. 3 to 1.[14] When the CO atmosphere was removed, both species disappeared, while **3** was reformed. Thus, **3** appeared to bind CO reversibly to form two isomers. The reaction was further probed using ^{13}CO, which eased ^{13}C NMR measurements. Seven ^{13}C signals corresponding to the acyl and carbonyl carbons were observed right after the introduction of ^{13}CO to a solution of **3** (Figure S1, SI).[14] The signals could be assigned to two species. The major species has δ(C) of 248.8, 208.5, 206.0, 204.8 ppm, while the minor species has δ(C) of 251.2, 214.3, 207.3 ppm. The two lowest-field signals are due to the acyl carbons, and their ratio of ca. 3:1 reflects the relative abundance of the two species. This ratio is in good agreement with that detected by ^{1}H NMR. Among the five signals for the CO ligands, three more intense signals should belong to

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the major species, while the other two belong to the minor species. Assuming that 6-coordinate tris(carbonyl) species are formed, we propose that the major species is the facial isomer and the minor species is the meridional isomer (**7A** and **7B**, Figure 3).[17] While there are two possible *mer*-isomers, only one of them exists, yet it could not be further identified.

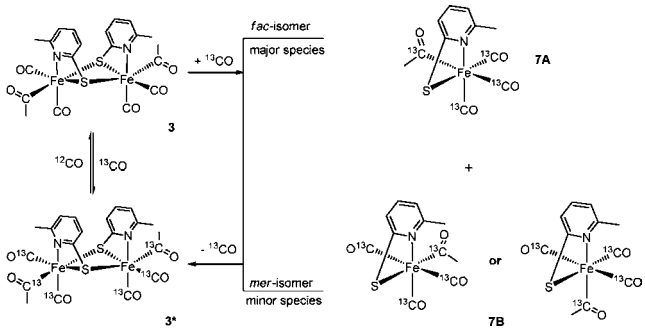

Figure 3. Reactivity of **3** with ^{13}CO and interconversion of **3** and **3***.

In the absence of CO, **7A** and **7B** quickly lost one CO to form **3***, an isotopologue of **3** (Figure 3).[14] According to ^{13}C NMR, all the acyl and carbonyl carbon atoms are ^{13}C labeled in **3***. In the IR spectra, all ν(CO) and ν(acyl) bands are shifted to lower frequencies in **3*** (Table S1).[14] Applying the Teller−Redlich product rule,[5,18] $\Pi(\nu^*/\nu) = (\mu/\mu^*)^{n/2}$, confirms that all acyl and carbonyl carbon are isotopically labeled - the experimental data give $\Pi(\nu^*/\nu) = 0.875$, which agrees with the value of 0.873 expected for six $^{13}C-O$ groups ($n = 6$). In a control experiment, **3*** was subjected to a charge/discharge cycle of ^{12}CO atmosphere. Two such cycles were sufficient to regenerate **3**.

The facile isotopic exchange among all the CO ligands in **3** suggests a fast equilibrium between **3** and **7A**+**7B** even under a CO atmosphere. The isotopic exchange of the acyl carbon with ^{13}CO is also rapid. Possible pathways are reversible elimination of CO and/or methyl migration from the acyl ligand. A number of Fe acyl poly(carbonyl) complexes have been previously reported.[13,15,19,20] Equilibrium between Fe methyl CO and Fe acyl was indeed observed.[20,21] For [Fe]-hydrogenase, however, no exchange between the extrinsic and intrinsic CO ligands was detected.[5] The rigidity of the internal Fe carbonyls in the enzyme might be attributed to two factors: (i) These Fe carbonyls are not labile, as they might be stabilized by interaction with the protein environment. This interaction might be the origin for the $\sim$20 cm^{-1} higher stretching frequencies for the CO and CN^- ligands in the CN-inhibited enzyme compared to those in model complex **5**. (ii) Unlike **3**, dimerization of the Fe active site is not possible in the protein. As for the isotopic exchange on the acyl ligand, no data are yet available for the enzyme.

Complex **3** did not activate H_2 (up to 50 bar).[14] This unreactivity is not unexpected because [Fe]-hydrogenase could not react with H_2 in the absence of the enzymatic substrate methenyl-H_4MPT^+.[3] Another factor to consider is the role of the secondary coordination sphere in [Fe]-hydrogenase. The homodimer **3** reacts as a masked form of its 5-coordinate monomer (**3′**). Even though **3′** reproduces the first coordination sphere of the active site of [Fe]-hydrogenase, it is missing a hydroxyl group at the α position to the pyridinyl nitrogen. The hydroxyl group may bear important functions in the enzyme. It could serve as an internal base, and it interacts with the side chain of His14.[8] Mutation of His14 reduced the activity of the wild-type enzyme to less than 1%.[6,8] The next generation of model compounds shall include similar secondary interactions.

In conclusion, we prepared an [$Fe^{II}Fe^{II}$] dithiolate complex (**3**), which reacted with small molecules to give monomeric Fe^{II} complexes that reproduce the first coordination sphere of the active site of [Fe]-hydrogenase and its CN^-- and CO-inhibited states. All carbonyl and acyl ligands in **3** undergo rapid isotopic exchange via monomeric Fe^{II} tricarbonyl intermediates.

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Supporting Information Available: Experimental details and crystallographic files for **3** and **6**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Vignais, P. M.; Billoud, B.; Meyer, J. *FEMS. Microbiol. Rev.* **2001**, *25*, 455–501. (b) Shima, S.; Thauer, R. K. *Chem. Rec.* **2007**, *7*, 37–46.
(2) Thauer, R. K.; Klein, A. R.; Hartmann, G. C. *Chem. Rev.* **1996**, *96*, 3031–3042.
(3) Vogt, S.; Lyon, E. J.; Shima, S.; Thauer, R. K. *J. Biol. Inorg. Chem.* **2008**, *13*, 97–106.
(4) (a) Lyon, E. J.; Shima, S.; Buurman, G.; Chowdhuri, S.; Batschauer, A.; Steinbach, K.; Thauer, R. K. *Eur. J. Biochem.* **2004**, *271*, 195–204. (b) Shima, S.; Lyon, E. J.; Sordel-Klippert, M. S.; Kauss, M.; Kahnt, J.; Thauer, R. K.; Steinbach, K.; Xie, X. L.; Verdier, L.; Griesinger, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 2547–2551. (c) Shima, S.; Lyon, E. J.; Thauer, R. K.; Mienert, B.; Bill, E. *J. Am. Chem. Soc.* **2005**, *127*, 10430–10435. (d) Korbas, M.; Vogt, S.; Meyer-Klaucke, W.; Bill, E.; Lyon, E. J.; Thauer, R. K.; Shima, S. *J. Biol. Chem.* **2006**, *281*, 30804–30813. (e) Pilak, O.; Mamat, B.; Vogt, S.; Hagemeier, C. H.; Thauer, R. K.; Shima, S.; Vonrhein, C.; Warkentin, E.; Ermler, U. *J. Mol. Biol.* **2006**, *358*, 798–809. (f) Guo, Y. S.; Wang, H. X.; Xiao, Y. M.; Vogt, S.; Thauer, R. K.; Shima, S.; Volkers, P. I.; Rauchfuss, T. B.; Pelmenschikov, V.; Case, D. A.; Alp, E. E.; Sturhahn, W.; Yoda, Y.; Cramer, S. P. *Inorg. Chem.* **2008**, *47*, 3969–3977.
(5) Lyon, E. J.; Shima, S.; Boecher, R.; Thauer, R. K.; Grevels, F. W.; Bill, E.; Roseboom, W.; Albracht, S. P. J. *J. Am. Chem. Soc.* **2004**, *126*, 14239–14248.
(6) Shima, S.; Pilak, O.; Vogt, S.; Schick, M.; Stagni, M. S.; Meyer-Klaucke, W.; Warkentin, E.; Thauer, R. K.; Ermler, U. *Science* **2008**, *321*, 572–575.
(7) Hiromoto, T.; Ataka, K.; Pilak, O.; Vogt, S.; Stagni, M. S.; Meyer-Klaucke, W.; Warkentin, E.; Thauer, R. K.; Shima, S.; Ermler, U. *FEBS Lett.* **2009**, *583*, 585–590.
(8) Hiromoto, T.; Warkentin, E.; Moll, J.; Ermler, U.; Shima, S. *Angew. Chem., Int. Ed.* **2009**, *48*, 6457–6460.
(9) (a) Volbeda, A.; Charon, M. H.; Piras, C.; Hatchikian, E. C.; Frey, M.; Fontecilla-Camps, J. C. *Nature* **1995**, *373*, 580–587. (b) Nicolet, Y.; Piras, C.; Legrand, P.; Hatchikian, C. E.; Fontecilla-Camps, J. C. *Struct. Fold. Des.* **1999**, *7*, 13–23. (c) Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science* **1998**, *282*, 1853–1858. (d) Armstrong, F. A. *Curr. Opin. Chem. Biol.* **2004**, *8*, 133–140.
(10) (a) Wang, X. F.; Li, Z. M.; Zeng, X. R.; Luo, Q. Y.; Evans, D. J.; Pickett, C. J.; Liu, X. M. *Chem. Commun.* **2008**, 3555–3557. (b) Obrist, B. V.; Chen, D. F.; Ahrens, A.; Schunemann, V.; Scopelliti, R.; Hu, X. L. *Inorg. Chem.* **2009**, *48*, 3514–3516. (c) Li, B.; Liu, T.; Popescu, C. V.; Bilko, A.; Darensbourg, M. Y. *Inorg. Chem.* **2009**, *48*, 11283–11289.
(11) Royer, A. M.; Rauchfuss, T. B.; Gray, D. L. *Organometallics* **2009**, *28*, 3618–3620.
(12) (a) Sadique, A. R.; Brennessel, W. W.; Holland, P. L. *Inorg. Chem.* **2008**, *47*, 784–786. (b) Tard, C.; Pickett, C. J. *Chem. Rev.* **2009**, *109*, 2245–2274.
(13) Mitsudo, T. A.; Ishihara, A.; Suzuki, T.; Watanabe, Y.; Masuda, H. *Organometallics* **1990**, *9*, 1357–1358.
(14) See Supporting Information.
(15) Smith, J. M.; Lachicotte, R. J.; Holland, P. L. *Organometallics* **2002**, *21*, 4808–4814.
(16) Kubas, G. J. *Chem. Rev.* **2007**, *107*, 4152–4205.
(17) Another possibility is that the two isomers are acyl rotamers. However, the rotamers should give rise to the same numbers of carbonyl ^{13}C NMR signals. Experimentally two signals were observed for the minor isomer, and three were observed for the major isomer. Therefore, this possibility is excluded, as peak overlapping is rare in ^{13}C spectra.
(18) Braterman, P. S. *Metal Carbonyl Spectra*; Academic Press: London, 1975.
(19) Shirasawa, N.; Nguyet, T. T.; Hikichi, S.; Moro-oka, Y.; Akita, M. *Organometallics* **2001**, *20*, 3582–3598.
(20) Bellachioma, G.; Cardaci, G.; Reichenbach, G. *J. Chem. Soc., Dalton Trans.* **1983**, 2593–2597.
(21) Pankowski, M.; Bigorgne, M. *J. Organomet. Chem.* **1983**, *251*, 333–338.

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