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An Iron Carbonyl Pyridonate Complex Related to the Active Site of the [Fe]-Hydrogenase (Hmd)

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A mononuclear iron bis(carbonyl) pyridonate complex (**1**), which exhibits several common structural features with the active site of the iron−sulfur cluster-free [Fe]-hydrogenase, was synthesized and characterized. Spectroscopic data of **1** suggests a 2+ oxidation state for the Fe ion in the [Fe]-hydrogenase. Complex **1** serves as a precursor to other hydrogenase models.

Three classes of hydrogenases are now known: the iron−sulfur cluster-containing [FeFe]-hydrogenase and [FeNi]-hydrogenase and the iron−sulfur cluster-free [Fe]-hydrogenase.[1,2] Phylogenetically unrelated, the three hydrogenases share common features. They all contain active sites made of first-row transition-metal ions (Fe, Ni) and sulfur, CO, and CN ligands.[3,4] The [FeFe]- and [FeNi]-hydrogenases catalyze hydrogen evolution and/or oxidation. [Fe]-hydrogenase, or the H_2-forming methylenetetrahydromethanopterin dehydrogenase (Hmd), catalyzes the reversible reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT$^+$) with H_2 to form methylenetetrahydromethanopterin (methylene-H_4MPT) and H^+ (Figure 1), an intermediary step in the reduction of CO_2 to methane by methanogens grown under nickel-limiting conditions.[5]

Compared to the prominent [FeNi]- and [FeFe]-hydrogenases, the newly discovered [Fe]-hydrogenase is unique. It is the only hydrogenase that requires one single metal (Fe) for function.[2,6] Spectroscopic measurements and especially two recent crystallographic studies[4,7] suggest that the Fe ion is coordinated to a cysteine S atom, two cis-arranged CO ligands, a yet unidentified ligand, and a pyridone molecule (Figure 1).[4,7−10] There is still ambiguity in the composition and binding mode of the pyridine ligand. According to the initial X-ray crystallographic study of the holoenzyme, the Fe ion is coordinated to the N atom of a 6-(carboxymethyl)-2-pyridone derivative that was identified as the light inactivation product of the Fe-containing cofactor.[4] However,

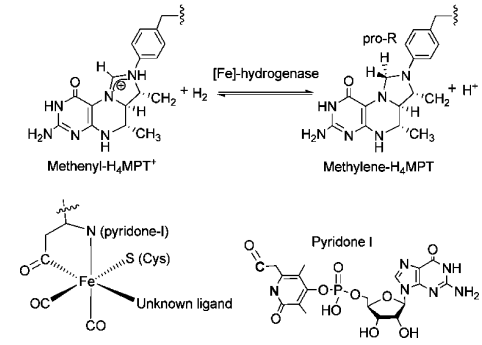

Figure 1. Top: Function of [Fe]-hydrogenase. The reaction is reversible with a $\Delta G°$ of −5.3 kJ/mol. Bottom left: Structure of the Fe-containing active site in [Fe]-hydrogenase. Bottom right: Structure for the pyridone I cofactor. The coordinating N and C atoms are highlighted in bold. Selected bond distances (Å): Fe−CO, 1.77; Fe−S, 2.34; Fe−N, 2.05.; Fe−C(acyl), 1.88. Bond distances are taken from the 2009 EXAFS study (ref 7), which revised the 2008 crystal structure (ref 4).

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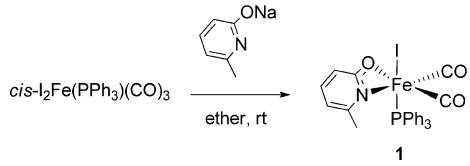

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Figure 2. Synthesis of complex **1**. The pyridone is shown as a mixture of both pyridone and pyridinol resonance forms; see the text for details.

subsequent work showed that the X-ray crystallographic and EXAFS data fit better with the structure in which a 6-(formylmethyl)-2-pyridone cofactor coordinates to the Fe ion via both its N and acyl C atoms (Figure 1).[7] Despite this uncertainty, the presence of such a six-membered-ring nitrogen ligand on a Fe center in a CO- and S-rich environment is unprecedented and intriguing.

Many useful model complexes for [FeNi]- and [FeFe]-hydrogenases are now available,[11] but few model complexes for [Fe]-hydrogenase are reported,[12,13] and none of them contains a mimic for the unusual pyridone cofactor. One uncertainty of [Fe]-hydrogenase is the oxidation state of the Fe center. Mössbauer and IR data suggested that the Fe ion can be either low-spin Fe^0 or Fe^{II}.[8,9] A recent study suggested 2+ oxidation state for the Fe ion, but the conclusion was made on an iron(II) bis(carbonyl) aminopyridinebis(thiolate) model complex lacking a pyridone-type ligand.[12] It is thus interesting to probe how a pyridone ligand may influence the electronic properties of the Fe ion. Surprisingly, there are only a few reported iron pyridone complexes, and none of them contains one pyridone ligand coordinating to one single Fe center.[14] Here we present a monomeric iron bis(carbonyl) pyridonate complex. The spectroscopic properties of the complex resemble those of [Fe]-hydrogenase and provide further evidence that the Fe ion in [Fe]-hydrogenase is Fe^{II}.

6-Methyl-2-pyridone was employed as a simple mimic of the pyridone cofactor found in the enzyme. The use of a triphenylphosphine coligand was critical to the stability of the resulting complex. Thus, the reaction of $Fe(CO)_3(PPh_3)I_2$[15] with sodium 6-methyl-2-pyridonate (L) yielded a crystalline solid (**1**; Figure 2). IR and NMR spectroscopic

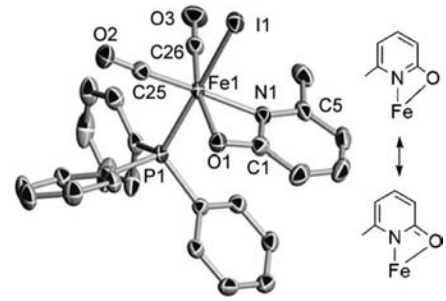

Figure 3. Solid-state structure of **1**. The iron−pyridone interaction is best described by two resonance forms as shown on the right. The thermal ellipsoids are displayed at 50% probability. Selected bond distances (Å) and angles (deg): Fe1−N1, 1.9612(19); Fe1−O1, 2.0106(14); Fe1−P1, 2.2652(6); Fe1−C25, 1.789(2); Fe1−C26, 1.768(2); Fe1−I1, 2.6450(3); C25−O2, 1.142(3); C26−O3, 1.140(3); C1−O1, 1.310(3); C1−N1, 1.351(3); C5−N1, 1.351(3); C25−Fe1−C26, 91.44(10); O1−Fe1−N1, 66.90(7).

data suggested a formula of $Fe(CO)_2(PPh_3)IL$ for **1**, which was confirmed by elemental analysis and X-ray crystallography.[16]

The solid-state structure of **1** shows that the Fe^{II} ion is in a six-coordinate, pseudo-octahedral ligand environment (Figure 3).[16] Two terminal CO ligands bind in a cis fashion, with an averaged Fe−C distance of 1.778(2) Å. The pyridonate ligand L is bidentate and coordinates to Fe via both the N and O atoms. The Fe−N and Fe−O distances are 1.9612(19) and 2.0106(14) Å, respectively. The C1−O1 and C1−N1 distances in L are between those of a single bond and a double bond.[17] The Fe−L fragment is therefore best described by a mixture of two resonance forms, in which L exists in either deprotonated pyridinol or deprotonated pyridone form (Figure 3).[18] The coordination sphere of Fe is completed by the axial iodide and triphenylphosphine ligands.

The structure of **1** and the active site of [Fe]-hydrogenase share some similarities. Both compounds contain two *cis*-carbonyls, and their Fe−CO distances are similar. Both contain a pyridone derivative. The pyridone ligand in the enzyme has an acyl group and coordinates to Fe via η^2-κ-N,C; the pyridone in **1** is unfunctionalized and binds Fe via η^2-κ-N,O. The Fe−N distances are comparable. Both pyridone rings are flat. In the crystallographic study of the holoenzyme, this arrangement was used as evidence for pyridinol from of the pyridone cofactor.[4] The structure of **1** suggests that the pyridone ring remains planar even if it contains some components of the pyridone tautomeric form.

In the IR spectrum of **1**, two intense absorption bands were observed with ν(max) of 2032 and 1987 cm^{-1}, revealing the presence of two terminal CO ligands (Figure S1 in the Supporting Information).[16] The intensities of the two CO bands are nearly equal, consistent with a cis configuration. This IR spectrum is similar to those of [Fe]-hydrogenase and its extracted Fe-containing cofactor, which show CO bands

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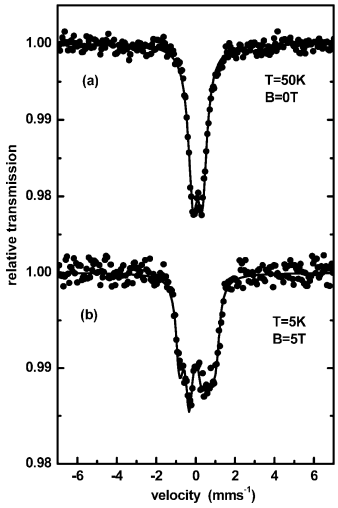

Figure 4. Mössbauer spectra of **1** obtained at $T = 50$ K (a) and at $T = 5$ K and an external field of $B = 5$ T applied perpendicularly to the γ-ray (b). The solid line represents a simulation assuming a diamagnetic ground state with parameters given in the text (except the line width used for the simulation displayed in part b, which was taken as 0.40 mm·s^{-1}).

at ν(max) = 2011 and 1944 cm^{-1} and 2031 and 1972 cm^{-1}, respectively.[8]

The average CO IR vibration frequencies can be correlated to the oxidation state of the Fe ions in monomeric iron bis(carbonyl) complexes.[12] For Fe^0, ν_{CO}(mean) = 1862 ± 23 cm^{-1}; for Fe^{II}, ν_{CO}(mean) = 2016 ± 22 cm^{-1}. ν_{CO}(mean) for [Fe]-hydrogenase is in the range for Fe^{II}; therefore, a 2+ oxidation state should be expected. However, the Fe ion is in an unusual ligand environment, so a derivation of its ν_{CO} from the known trend is also possible. ν_{CO}(mean) for complex **1** (2009 cm^{-1}) agrees with the norm for other iron(II) bis(carbonyl) complexes, suggesting that coordination of a pyridone ligand to a iron(II) bis(carbonyl) fragment does not result in a drastic change in the CO vibration frequencies. This reassures, albeit does not prove, the judgment from the IR study that the Fe center in [Fe]-hydrogenase is Fe^{II}.[8,12,19]

The Mössbauer spectrum of the Fe^{II} model complex **1** displayed in Figure 4a shows a symmetric quadrupole doublet with an isomer shift $\delta = 0.10(2)$ mm·s^{-1}, a quadrupole splitting $\Delta E_Q = 0.48(2)$ mm·s^{-1}, and a line width of $\Gamma = 0.57$ mm·s^{-1}. Field-dependent Mössbauer studies of [Fe]-hydrogenase found $\delta = +0.06$ mm·s^{-1} and a positive quadrupole splitting ($\Delta E_Q = +0.65$ mm·s^{-1}) for the diamagnetic Fe center.[9] Whereas these Mössbauer parameters are consistent with a low-spin Fe^{II} or Fe^0 center, the very low isomer shift seems to favor Fe^0.[9] However, it was recently shown that a iron(II) bis(carbonyl) bis(thiolate) complex exhibits a similarly low isomer shift.[12]

The Mössbauer spectrum of **1** obtained in an external field of 5 T (Figure 4b) shows that **1** also has a diamagnetic ground state as well as a positive sign of the quadrupole splitting. The asymmetry parameter of the electric field gradient used for the simulation displayed in Figure 4b has been taken as $\eta = 0$. The δ value of **1** and that of [Fe]-hydrogenase are very much comparable within the experimental accuracy of ±0.02 mm·s^{-1}. Also the positive sign of ΔE_Q is found in both cases, and the η values are comparable but the ΔE_Q values differ slightly. However, the free active Fe-containing cofactor of the protein itself exhibits $\Delta E_Q = 0.43$ mm·s^{-1}, which is in very good agreement with the ΔE_Q value of **1**.[9] Thus, this work suggests that the Mössbauer parameters of the Fe ion in [Fe]-hydrogenase could arise from a Fe^{II} center in an octahedral environment with a pyridone and two CO ligands.

The redox properties of **1** were measured by cyclic voltammetry. **1** undergoes one nonreversible and one quasi-reversible reduction at −1.5 and −1.75 V in CH_2Cl_2 vs ferrocene/ferrocenium, respectively (Figure S2 in the Supporting Information).[16] They are tentatively assigned to the $Fe^{II/I}$ and $Fe^{I/0}$ couples.

Complex **1** serves as a precursor to other synthetic mimics of [Fe]-hydrogenase. A preliminary study shows that the salt metathesis reaction of **1** with PhSNa gave a mixture of complex $Fe(CO)_2(PPh_3)(SPh)L$ (**2**), free PPh_3, and one or more paramagnetic species.[16] Complex **2** is highly unstable, precluding purification by recrystallization. However, the reaction of **1** with sodium 2,6-dimethylphenylthiolate gave complex $Fe(CO)_2(PPh_3)\{S(2,6\text{-}Me_2C_6H_3)\}L$ (**3**), which could be purified and isolated. Elemental analysis and ^{1}H and ^{31}P NMR and IR spectroscopic data are consistent with the formula of **3**.[16,20]

In summary, we have prepared a monomeric iron bis(carbonyl) pyridonate complex as a synthetic model for the active site of [Fe]-hydrogenase. Spectroscopic studies of **1** complement earlier work on the enzyme and other model compounds and support the proposal that the Fe ion in [Fe]-hydrogenase exists as Fe^{II}. Reactions of **1** lead to new [Fe]-hydrogenase model compounds.

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Supporting Information Available: Experimental details, spectroscopic data, and a CIFfile for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) As one reviewer pointed out, the IR frequencies also depend on the charge.

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