Bioinorganic Chemistry

A Trinuclear [NiFe] Cluster Exhibiting Structural and Functional Key Features of [NiFe] Hydrogenases**

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Hydrogenases are enzymes that control formation and consumption of protons and electrons by catalyzing of the reversible H_2 oxidation [Eq. (1)]. Therefore, they play a central role in the natural hydrogen and energy metabolism.[1]

$$H_2 \overset{\text{hydrogenase}}{\rightleftharpoons} 2\,H^+ + 2\,e^- \qquad (1)$$

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Hydrogenases are essential for many microorganisms which receive energy by reduction of CO_2, carbonates, sulfates, or nitrates.[2] These enzymes are of great biotechnological interest[3] owing to their capability to produce molecular hydrogen.[4] [NiFe] hydrogenases are the most abundant of the four types, the [NiFe], [NiFeSe], Fe-only, and metal-free hydrogenases.[3,5] Figure 1 depicts the active center of hydrogenase from *Desulfovibrio gigas*.

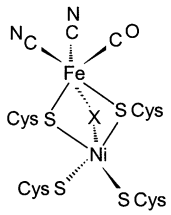

Figure 1. Schematic structure of the active center from *D. Gigas* hydrogenase in the oxidized form ("X" represents a bridging group, probably OH^- or O^{2-}).[6]

Whereas all hydrogenases perform the H_2 production/consumption reversibly, the [NiFe] hydrogenases generally have the greatest reaction rates for H_2 consumption and the "Fe-only" hydrogenases for H_2 production.[7] Not all [NiFe] hydrogenases obey to this rule. Some of them are known to be more active in catalyzing H_2 production than H_2 oxidation.[8]

The mechanism of the redox reaction is still poorly understood. [NiFe] hydrogenases exist in numerous redox states designated Ni-A, Ni-B, Ni-SU, Ni-SI, Ni-C, and Ni-R that can be labeled by their EPR spectra and individual ν(CO) frequencies.[3,9] The relationship of these redox states to the metal oxidation states is still unknown. Furthermore, it is not known where the H_2 molecule is activated and turned over and why two different metals are needed in [NiFe] hydrogenases. To shed light on these questions, several di- and oligonuclear compounds have been synthesized by various research groups.[10] Despite this research the number of compounds containing sulfur bridged Ni and Fe centers remains very small.[11]

We recently reported the complex [('S_2')Ni-μ('S_3')Fe(CO)(PMe_3)$_2$] ('S_2'$^{2-}$ = 1,2-benzenedithiolate(2−), 'S_3'$^{2-}$ = bis(2-mercaptophenyl)sulfide(2−)).[12] This complex is currently the closest approach to the active site of [NiFe] hydrogenases since nickel and iron are bridged by two thiolate donors and a carbonyl ligand is linked to the iron center. Nevertheless, it can only serve as a structural model compound for the active site as it does not show hydrogenase activity. This is also the case in the other structural model compounds for [NiFe] hydrogenases reported to date.

Herein, we report the synthesis of [('S_2')$\{$Ni(PMe_3)$\}_2$Fe(CO)('S_2')$_2$] (**1**), the first trinuclear [NiFe] cluster that shows structural (iron and nickel centers in sulfur dominated coordination spheres, iron bound CO) as well as functional properties of the active site of [NiFe] hydrogenases and, therefore, serves as the first structural and functional model. The trinuclear [NiFe] cluster **1** resulted from the reaction of [$\{$Fe(CO)$_2$('S_3')$\}_2$] with labile [Ni(PMe_3)$_2$('S_2')] according to Equation (2) in 19% yield. Monitoring the ν(CO) frequency by IR spectroscopy showed that in addition to **1** several other CO-containing complexes formed as byproducts on which we recently reported.[12]

The trinuclear cluster **1** consists of a square-pyramidal [Fe(CO)('S_2')$_2$] fragment which is connected by thiolate bridges to two [Ni(PMe_3)] fragments that are linked through

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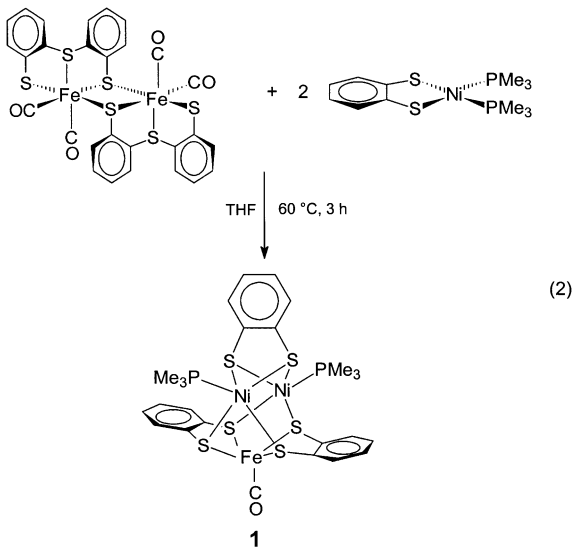


(2)

1

thiolates. With an average Ni–Fe separation of 259.6(1) pm and a Ni–Ni separation of 240.5(1) pm all metal atom positions are very close to each other so that there are not only M-S-M bridging but also metal–metal interactions. The Fe–S bond (av. 221.9(1) pm, see Figure 3) in the $\{Fe(CO)(`S_2')_2\}$ unit and the Ni–S bond lengths (av. 230.4(1) pm) in the $\{\mu\text{-}(`S_2')\{Ni(PMe_3)\}_2\}$ fragment are shorter than the Ni–S bonds in between these units (av. 237.0(1) pm). Although $[Ni(`S_2')_2\{Ni(PPh_3)\}_2(`S_2')]$, a complex very similar to **1** in which the Fe-CO fragment is replaced by an Ni atom, was reported a few years ago,[13] no information is available on its redox properties.

As hydrogenases catalyze the reversible interconversion of protons and electrons into molecular dihydrogen and consequently show redox activity electrochemical studies of model hydrogenase compounds are essential. Figure 2 shows the cyclic voltammogram of **1**. The three quasireversible redox waves at −1071 mV, −95 mV, and 844 mV are assigned the redox couples $\mathbf{1}^{0/-}$, $\mathbf{1}^{0/+}$, and $\mathbf{1}^{+/2+}$, respectively. The redox potential of the redox wave at −95 mV ($\mathbf{1}^{0/+}$) lies within a potential range observed for several typical [NiFe] hydrogenase redox reactions.[3,14] This encouraged us to treat **1** with HBF_4 and to monitor this reaction by IR spectroscopy, the ν(CO) vibration serving as a probe for the cluster electron density. In CH_2Cl_2 the corresponding band at 1916 cm^{-1} is shifted to 1976 cm^{-1} upon addition of HBF_4. After subsequent treatment with NBu_4BH_4 the original band reappeared at 1916 cm^{-1}. When solutions of **1** in CH_2Cl_2 were combined with one equivalent of ferrocenium salt $[Cp_2Fe][PF_6]$ (a one-electron oxidant ($Cp = C_5H_5$) the ν(CO) band was shifted again to 1976 cm^{-1} These findings confirmed that the shift of 60 cm^{-1} corresponds to single-electron-transfer steps. The corresponding EPR spectra of the cationic compounds prepared with HBF_4 and ferrocenium salts exhibit identical singlets at room temperature ($\langle g\rangle = 2.092$). The glass-spectra consist of a broad singlet at almost the same position ($\langle g\rangle =$ 2.087) and a rhombic spectrum of low intensity ($g_1 = 2.181$, $g_2 = 2.054$, $g_3 = 2.025$), which could not be related unambiguously to the molecular structure of the paramagnetic complex cation.

Scheme 1 depicts the redox reactions of **1** with oxidants and reductants. In an NMR tube a solution of **1** in CD_2Cl_2 was treated with HBF_4. Monitoring the reaction by ^1H NMR spectroscopy showed signals of a diamagnetic species at $\delta =$ 7.28, 6.71, 6.57, 6.22, and 2.09 ppm before the addition of HBF_4. After the addition, owing to the paramagnetism of $\mathbf{1}^+$, these signals vanished and a sharp signal at $\delta = 4.6$ ppm appeared which was assigned to free dihydrogen. After addition of NBu_4BH_4 the signals of the diamagnetic **1** reappeared and as a result of the oxidation of borohydride to dihydrogen and diborane the singlet arising from free dihydrogen gains in intensity. When oxidized by $[Cp_2Fe][PF_6]$, the ion $\mathbf{1}^+$ proved to be stable in solution over several weeks, whereas oxidation through HBF_4 led to a decrease of the ν(CO) frequency of about 25 % after 24 h. Thus, the species resulting from oxidation with the ferrocenium salt could be crystallized and an X-ray structure determination could be performed (Figure 3).[15]

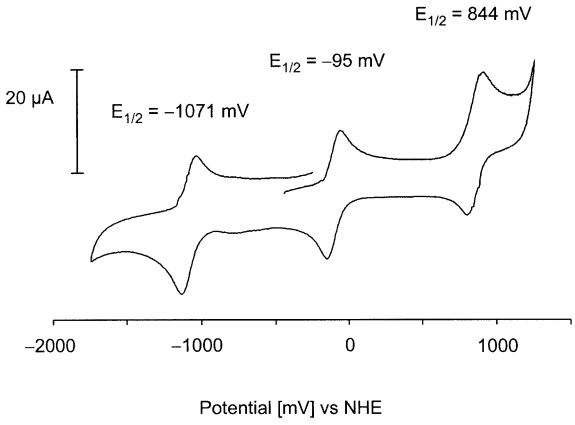
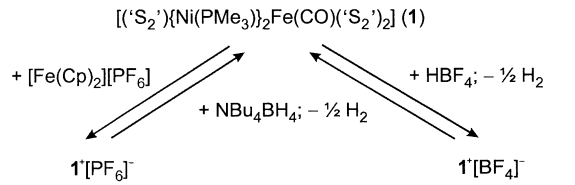


Figure 2. Cyclic voltammogram of **1** in CH_2Cl_2. NHE = normal hydrogen electrode.



Scheme 1. Redox activity of **1** with HBF_4 and $[Cp_2Fe][PF_6]$. The resulting ion $\mathbf{1}^+$ was then reduced with NBu_4BH_4.

It is anticipated that many oxidoreductases undergo no or only marginal structural changes while redox reactions occur. The structural rigidity means a low reorganization barrier and therefore favors rapid electron transfer.[16] This criterion would fit to the redox pair $\mathbf{1}/\mathbf{1}^+$ because no significant changes in bond lengths or angles take place. So the average Fe–Ni separation before oxidation is 259.6(1) pm and afterwards 258.2(2) pm, the Ni–Ni bond length in **1** changes from 240.5(1) pm to 243.1(2) pm, and the average Fe–S separation from 221.9(1) pm to 220.5(6) pm after oxidation. Even the

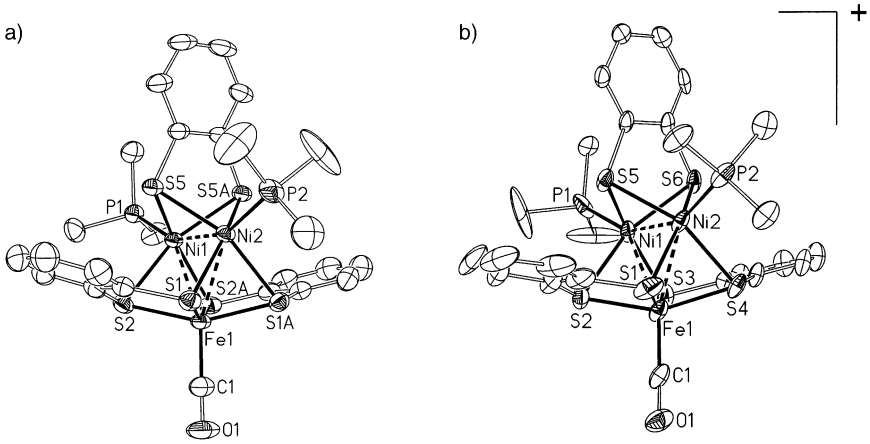


Figure 3. Molecular structures of **1** (a) and $\mathbf{1}^+$ (b; H atoms omitted). Selected interatomic distances [pm] and angles [°] of **1**: Fe1-S1 222.0(1), Fe1-S2 221.8(1), Fe1-C1 173.8(5), Fe1···Ni1 262.3(1), Fe1···Ni2 256.9(1), Ni1···Ni2 240.5(1), Ni1-S2 236.1(1), Ni2-S1 237.9(1), Ni1-P1 221.3(2), Ni2-P2 219.3(2), Ni1-S5 230.4(1), Ni2-S5 232.4(1); S1-Fe1-S2 90.00(4), S1-Fe1-S1A 84.01(5), S1-Fe1-S2A 154.24(5), Ni2-Fe1-Ni1 55.18(3), Ni2-Ni1-Fe1 61.27(3), Ni1-Ni2-Fe1 63.54(3). Selected interatomic distances [pm] and angles [°] of $\mathbf{1}^+$: Fe1-S1 220.4(2), Fe1-S2 219.7(2), Fe1-C1 174.4(8), Fe1···Ni1 258.8(2), Fe1···Ni2 257.7(2), Ni1···Ni2 243.1(2), Ni1-S2 232.8(2), Ni2-S1 232.3(2), Ni1-P1 220.8(2), Ni2-P2 221.7(2), Ni1-S5 229.3(2), Ni2-S5 229.0(2); S1-Fe1-S2 91.37(8), S1-Fe1-S4 83.33(8), S1-Fe1-S3 154.17(9), Ni2-Fe1-Ni1 56.14(3), Ni2-Ni1-Fe1 61.70(4), Ni1-Ni2-Fe1 62.15(4).

Ni–S bond lengths of the Ni-S-Ni bridges change only marginally from 230.4(1) pm to 229.6(2) pm after oxidation. The average Ni–S bond of the Ni-S-Fe bridges shortens a little after oxidation from 237.0(1) pm to 233.1(9) pm. The Ni-Fe-Ni angle increases from 55.18(3)° in the neutral compound **1** to 56.14(3)° in the ion $\mathbf{1}^+$. In summary cluster **1** combines structural properties of the [NiFe] hydrogenases active site, hydrogenase reactivity, and structural rigidity during electron transfer processes.

Experimental Section

All manipulations were carried out in absolute solvents under exclusion of air. $[\{Fe(CO)_2(\text{'}S_3\text{'})\}_2]$ and $[Ni(PMe_3)_2(\text{'}S_2\text{'})]$ were prepared by literature methods.[12,17] IR spectroscopy: Perkin Elmer 983, 1620 FT-IR and 16PC FT-IR; NMR spectroscopy: Lambda LA 400; EPR: Bruker ESP 300E spectrometer; mass spectrometry: JEOL MSTATION 700; elemental analysis: Carlo Erba EA 1106 or 1108.

1: A THF suspension (40 mL) of $[Fe(CO)_2(\text{'}S_3\text{'})]_2$ (535 mg, 0.74 mmol) and $[Ni(PMe_3)_2(\text{'}S_2\text{'})]$ (523 mg, 1.49 mmol) was heated to 60 °C for 3 h. MeOH (80 mL) was added to the resulting dark red to black solution. Black-grey microcrystals precipitated and were removed from the mother liquor after 48 h and washed with MeOH (20 mL). Yield: 215 mg (19 %). ^{1}H NMR (399.7 MHz, $[D_8]$THF): $\delta =$ 7.28 (m, 4H, C_6H_4), 6.71 (m, 4H, C_6H_4), 6.57 (m, 2H, C_6H_4), 6.22 (m, 2H, C_6H_4), 2.09 ppm (s, 18H, PC_3H_9); $^{13}C\{^1H\}$ NMR (100.4 MHz, CD_2Cl_2): $\delta = 207.3$ (CO), 146.9, 130.9, 130.3, 125.1, 123.3, (C_6H_4), 18.3 ppm (PCH_3); $^{31}P\{^1H\}$ NMR (161.7 MHz, CD_2Cl_2): $\delta = 214.7$ ppm (br, PC_3H_9); IR (KBr): $\tilde{\nu} = 1910\ cm^{-1}$ (CO)); IR (CH_2Cl_2): $\tilde{\nu} = 1916\ cm^{-1}$ (CO); MS (FD, $[D_8]$THF): $m/z = 774$ ($[Ni_2Fe(CO)(PMe_3)_2(\text{'}S_2\text{'})_3]^+$); elemental analysis calcd (%) for $C_{25}H_{30}FeNi_2OP_2S_6$: C 38.79, H 3.91, S 24.85; found: C 38.58, H 3.79, S 24.86.

$\mathbf{1}^+[PF_6]^-$: $[Fe(Cp)_2][PF_6]$ (14 mg, 0.04 mmol) was added to a CH_2Cl_2 solution (5 mL) of **1** (34 mg, 0.04 mmol). Evaporation of solvent yielded a brown powder. IR (KBr): $\tilde{\nu} = 1951\ cm^{-1}$ (CO); IR (CH_2Cl_2): $\tilde{\nu} = 1976\ cm^{-1}$ (CO); EPR (9.43 GHz, CH_2Cl_2): 300 K: $\langle g \rangle = 2.092$ (s); 135 K: $g = 2.181, 2.087, 2.054, 2.025$; MS (FD, CH_2Cl_2): $m/z = 774$ ($[Ni_2Fe(CO)(PMe_3)_2(\text{'}S_2\text{'})_3]^+$); single crystals suitable for X-ray structure determination were obtained by slow diffusion of *n*-pentane in a saturated CH_2Cl_2 solution of $\mathbf{1}^+[PF_6]^-$ at 10 °C; some of the crystals were crushed and dried in vacuum; elemental analysis calcd (%) for $C_{25}H_{30}FeNi_2OP_3S_6F_6$: C 32.67, H 3.29, S 20.93; found: C 32.93, H 3.27, S 20.83.

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[15] X-ray structure analysis of **1**·2THF: Black prisms of **1** were obtained from a THF solution. A suitable single crystal was embedded in protective perfluoropolyether oil. Data were collected at 210 K on a Siemens P4 diffractometer using $Mo_{K\alpha}$ radiation ($\lambda = 71.073$ pm), graphite monochromator, and the ω-scan technique. Lorentz, polarization, and absorption corrections (Psi-scans, $T_{min} = 0.090$, $T_{max} = 0.139$) were applied. $C_{33}H_{46}FeNi_2O_3P_2S_6$, crystal size $0.62 \times 0.56 \times 0.24$ mm, monoclinic space group *Cm* (no. 8), $a = 1044.7(2)$, $b = 1872.0(4)$, $c = 1016.2(2)$ pm, $\beta = 97.80(2)°$, $V = 1.9690(7)$ nm^3, $Z = 2$, $\rho_{calcd} = 1.549$ g cm^{-3}, $\mu = 1.742$ mm^{-1}, 4666 measured reflections ($4.0 < 2\theta < 54.0°$), 4431 unique reflections, 4223 observed reflections [$I > 2\sigma(I)$], 272 parameters, $wR_2 = 0.0796$ (all data), $R_1 = 0.0301$ [$I > 2\sigma(I)$]. The structure was solved by direct methods and refined by full-matrix least-squares procedures on F^2 using SHELXTL NT 6.12. All non-hydrogen atoms have been refined anisotropically. The complex molecule is situated on a crystallographic mirror plane resulting in *Cm* symmetry. The compound crystallizes with two molecules THF per formula unit. The solvent molecules are disordered, two alternative sites could be refined giving site occupancy factors of 0.5 each. All hydrogen atoms were geometrically positioned. X-ray structure analysis of **1**-PF_6·1.33 CH_2Cl_2: Black plates of **1**-PF_6·1.33 CH_2Cl_2 were obtained from a CH_2Cl_2/*n*-pentane mixture. A suitable single crystal was embedded in protective perfluoro-polyether oil. Data were collected at 100 K on a Bruker-Nonius KappaCCD diffractometer using $Mo_{K\alpha}$ radiation ($\lambda = 71.073$ pm) and a graphite monochromator. Images were taken using ϕ- and ω-rotations with a rotation angle of 1.1° and an irradiation time of 55 s per frame. Lorentz, polarization, and semiempirical absorption corrections (SADABS using multiple scans, $T_{min} = 0.798$, $T_{max} = 1.000$) were applied. $C_{26.33}H_{32.67}Cl_{2.67}F_6FeNi_2OP_3S_6$, crystal size $0.40 \times 0.30 \times 0.06$ mm, monoclinic space group *C*2/*c* (no. 15), $a = 5543.2(3)$, $b = 1048.8(2)$, $c = 2051.7(2)$ pm, $\beta = 104.056(6)°$, $V = 11.571(2)$ nm^3, $Z = 12$, $\rho_{calcd} = 1.778$ g cm^{-3}, $\mu = 2.025$ mm^{-1}, 49275 measured reflections ($7.0 < 2\theta < 52.8°$), 11049 unique reflections, 6677 observed reflections [$I > 2\sigma(I)$], 890 parameters, $wR_2 = 0.1433$ (all data), $R_1 = 0.0656$ [$I > 2\sigma(I)$]. The structure was solved by direct methods and refined by full-matrix least-squares procedures on F^2 using SHELXTL NT 6.12. All non-hydrogen atoms have been refined anisotropically. The asymmetric unit contains 1.5 molecules of $\mathbf{1}^+$. Half a molecule is situated on crystallographic twofold axis resulting in strong disorder. No hydrogen atoms have been included for this strongly disordered cation. Disorder is also observed for one of the PF_6^- counterions and the CH_2Cl_2 solvate molecules. A number of crystals has been investigated, all of them exhibiting the same type of disorder. Hydrogen atoms for the non-disordered cation and the solvate molecules were geometrically positioned. CCDC 224468 (**1**) and CCDC 224469 ($\mathbf{1}^+$) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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