FULL PAPER

Dalton
www.rsc.org/dalton

Targeting synthetic analogues of the metallo-sulfur active sites of nickel enzymes capable of important catalysis †

Matt C. Smith, J. Elaine Barclay, Sian C. Davies, David L. Hughes and David J. Evans *
Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Colney, Norwich, UK NR4 7UH. E-mail: dave.evans@bbsrc.ac.uk

Received 23rd June 2003, Accepted 15th July 2003
First published as an Advance Article on the web 22nd September 2003

The nickel containing enzymes NiFe-hydrogenase, carbon monoxide dehydrogenase and acetyl-CoA synthase are able to catalyse environmentally, and potentially industrially, important reactions: hydrogen uptake; carbon dioxide/ carbon monoxide interconversion; and incorporation of carbon monoxide to form acetyl-CoA, respectively. Progress toward the synthesis of nickel–iron complexes with structural features related to the active sites of these enzymes are described, together with the synthesis, properties and crystal structure of the first methylated-nickel–iron diheterometallic complex, $[\{Fe(\mathit{N}S_3)(NO)\text{-}\mathit{S}\}Ni(CH_3)(dppe)]$ ($\mathit{N}S_3 = N(CH_2CH_2S)_3{}^{3-}$), relevant to a proposed mechanistic intermediate in the acetyl-CoA synthase mechanism.

Introduction

Enzymes can catalyse reactions which are very relevant to clean technologies. Of particular interest are the hydrogenases and nickel-containing carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS). Hydrogenases catalyse the interconversion of hydrogen and protons/electrons. The bifunctional enzyme CODH/ACS converts carbon dioxide through to, eventually, cell carbon. Understanding the underlying chemistry at the enzyme active sites (which utilise abundant iron and/or nickel and sulfur) may inform the design of novel, cheaper, "greener" catalysts for hydrogen production or uptake, for carbon dioxide sequestration and for alternative routes for the synthesis of chemical feedstocks.

The hydrogenases can be classified into three main groups: all-iron hydrogenases which are bidirectional; nickel–iron hydrogenases which are generally involved in hydrogen uptake; and transition metal free hydrogenases. X-ray crystallographic structures of all-iron hydrogenases from *Desulfovibrio desulfuricans* and *Clostridium pasteurianum*,[1,2] together with spectroscopic data on all-iron hydrogenase from *D. vulgaris*,[3] show that the active site is an Fe_4S_4-cluster linked by a bridging cysteinyl sulfur to an "organometallic" Fe_2S_3 sub-site (Fig. 1a). Recently, considerable advances have been made in the synthesis of synthetic analogues of the Fe_2S_3 sub-site.[4]

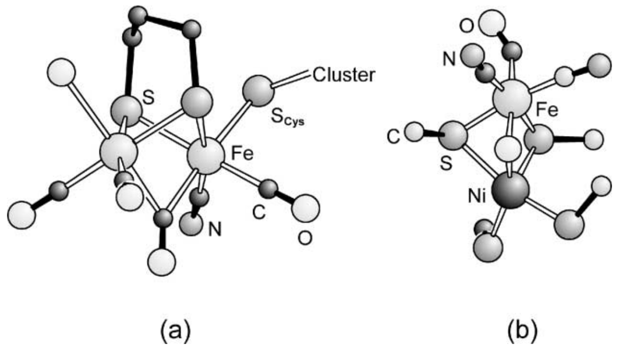

Fig. 1 (a) Composite representation of the "organometallic" Fe_2S_3 sub-site of all-iron hydrogenase; (b) The structure of the NiFe-site in inactive, as-isolated NiFe-hydrogenase.

The nickel–iron hydrogenase from *D. gigas*, as aerobically isolated in the inactive form, has been characterised by X-ray crystallography.[5] The active site, where dihydrogen is converted to protons and electrons, is a dinuclear thiolate-bridged nickel–iron complex. The nickel atom is coordinated by four cysteinate-sulfur atoms, two of which bridge to the iron atom. The other ligands to iron, as shown by crystallography and spectroscopy,[5,6] are cyanide and carbon monoxide. In the aerobically isolated crystals there is an additional bridging feature, probably oxo or hydroxo, which is not present in the active form of the enzyme (Fig. 1b). Several other structures of NiFe-hydrogenase have since been reported.[7]

Recently, crystal structures of a bifunctional CODH/ACS protein from *Moorella thermoacetica* have been reported and they show two distinct active sites: one at which carbon dioxide and carbon monoxide are interconverted, Cluster C; and one where carbon monoxide is utilised in the acetylation of coenzyme A to acetyl-CoA, Cluster A.[8,9] In the structure at 2.2 Å resolution by Drennan *et al.*,[8] Cluster C is an asymmetric $NiFe_4S_5$ assembly. The nickel is approximately four-coordinate square planar and one of the iron atoms is extraneous to the cuboidal-like core (Fig. 2a). This assembly has been previously observed in the 1.6 Å resolution structure of the dithionite-reduced state of CODH from *Carboxydothermus hydrogenoformans*.[10] Cluster C of CODH from *Rhodospirillum*

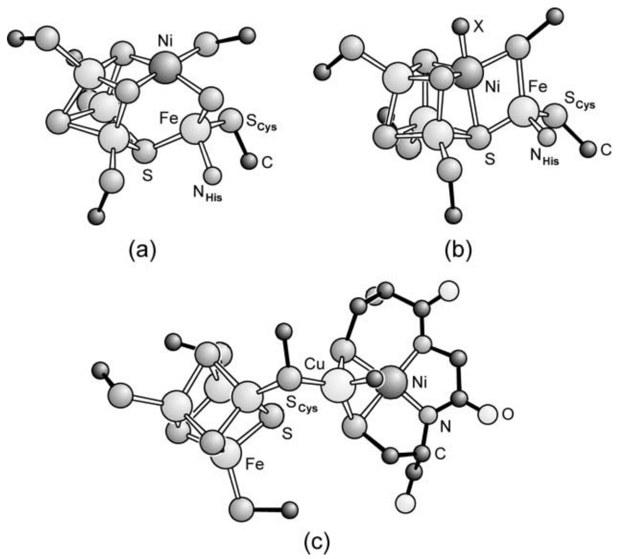

Fig. 2 The metal–sulfur assemblies of (a) reduced Cluster C of CODH from *M. thermoacetica*;[10] (b) CO-preincubated Cluster C of CODH from *R. rubrum*, X is an unidentified ligand, possibly non-substrate CO;[11] (c) Cluster A of ACS from *M. thermoacetica* from Drennan *et al.*[8]

---

† Based on the presentation given at Dalton Discussion No. 6, 9–11th September 2003, University of York, UK.

DOI: 10.1039/b307175h

rubrum preincubated with carbon monoxide has a similar structure,[11] Fig. 2b, except that the cuboidal fragment is "closed" and the nickel is five-coordinate in approximately a trigonal bipyramidal pattern. The fifth ligand, X, is, possibly, non-substrate carbon monoxide. This structure has some similarity to that of Cluster C observed by Fontecilla-Camps *et al.* in the 1.9 Å resolution structure of CO-treated CODH/ACS.[9]

Cluster A has a totally unexpected structure and is a remarkable assembly of an Fe_4S_4 cubane-cluster bridged through cysteinyl sulfur to a binuclear metal subsite.[8,9] In the structure of Drennan *et al.*[8] this subsite comprises copper and nickel (Fig. 2c). In the structure by Fontecilla-Camps *et al.*[9] the copper is substituted by either a zinc or nickel. The physiologically important combination of metal atoms at this active site remains controversial.[8,9,12,13] Previously, it had been proposed that both Cluster A and C were far simpler assemblies based on a Fe_4S_4-cluster linked through cysteinyl sulfur to a nickel–iron[14] or nickel based fragment,[15] respectively. The ACS reaction (the synthesis of acetyl-CoA by the non-redox condensation of a methyl group, a carbonyl group and an organic thiol) parallels the industrial Monsanto modification of the Reppe process for the production of acetic acid, both apparently involving metal carbonyl, methyl-metal and acyl-metal intermediates.

We have utilised the anions $[Fe(NS_3)(CO)]^-$ and $[Fe(NS_3)(NO)]^-$ $\{NS_3 = N(CH_2CH_2S)_3{}^{3-}\}$[16,17] as chelate ligands to nickel to generate materials with structural features of relevance to the active sites of these nickel enzymes. For example, the crystallographically characterised NiFe-dinuclear complexes $[\{Fe(NS_3)(CO)_2\text{-}S,S'\}NiCl(dppe)]$[18] **1** and $[\{Fe(NS_3)(NO)\text{-}S,S'\}NiCl(dppe)]$[19] **2**, Fig. 3, have a core structure similar to that of the active site of the NiFe-hydrogenase, Fig. 4. The core of each is dinuclear with nickel bound to iron by a bis(thiolate-bridge) and the iron atom is also bound by diatomic molecules. The trinuclear complex $[Ni\{Fe(NS_3)(CO)\text{-}S,S'\}_2]$[19,20] **3**, that unusually possesses nickel(II) coordinated in an almost regular tetrahedral geometry by thiolate-sulfur, has some structural features relevant to the active site structures of CODH/ACS. Here we report progress toward improved structural analogues of the active site of NiFe-hydrogenase, in which nickel is in an all-sulfur environment, and the preparation and crystal structure of $[\{Fe(NS_3)(NO)\text{-}S\}Ni(CH_3)(dppe)]$. This complex can be considered a chemical model of a proposed[9,13,14] nickel-methyl intermediate in the catalytic cycle of ACS and is the first example of a methylated-nickel in a heterodimetallic assembly.

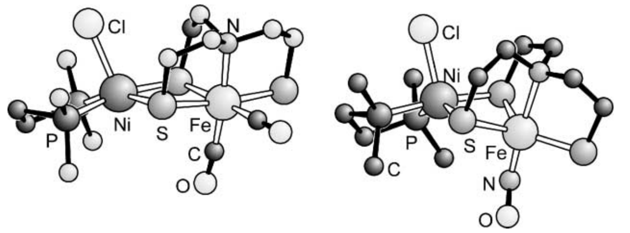

Fig. 3 The structures of NiFe-dinuclear complexes $[\{Fe(NS_3)(CO)_2\text{-}S,S'\}NiCl(dppe)]$ **1** (left) and $[\{Fe(NS_3)(NO)\text{-}S,S'\}NiCl(dppe)]$ **2** (right). The phenyl groups of the dppe ligand and all hydrogen atoms have been omitted for clarity.

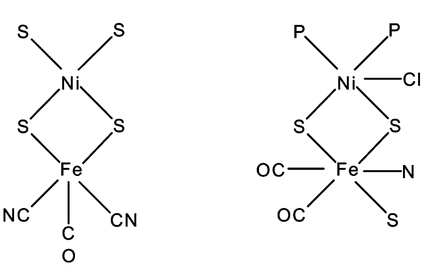

Fig. 4 A schematic comparison of the proposed, active NiFe-hydrogenase active centre (left) and the core of complex **1** (right).

Results and discussion

Nickel in an all-sulfur environment

To improve the structural analogy of complexes **1** and **2** to the active site of NiFe-hydrogenase it is desirable to replace the diphosphine ligation by sulfur ligation. An alternative, higher yield, preparation of the trinuclear complex $[Ni\{Fe(NS_3)(CO)\text{-}S,S'\}_2]$ **3** is by reaction of $[Fe(NS_3)(CO)]^-$ with $[NiCl_2(PPh_3)_2]$. Both the chloride and monophosphine ligands to nickel are labile in this system. Subsequent to isolation of **3** from this reaction mixture other products could be isolated: $[NEt_4][NiCl_3(PPh_3)]$, the structure of which we have reported elsewhere,[21] triphenylphosphine and tetraethylammonium chloride. The overall stoichiometry of the reaction is represented in eqn. (1).

$$2[NEt_4][Fe(NS_3)(CO)] + 2[NiCl_2(PPh_3)_2] \longrightarrow [Ni\{Fe(NS_3)(CO)\text{-}S,S'\}_2] + [NEt_4][NiCl_3(PPh_3)] + [NEt_4]Cl + 3PPh_3 \tag{1}$$

When the reaction is performed in the presence of one equivalent of ethanedithiol, under an atmosphere of carbon monoxide, a dark red solution, consistent with **3**, is formed immediately but on stirring this quickly turns brown. From the reaction mixture can be isolated a brown, insoluble, solid which has an elemental analysis consistent with the formulation $[\{Fe(NS_3)(CO)\text{-}S,S'\}Ni(SCH_2CH_2S)]$ **4**. The Mössbauer parameters are consistent with the iron remaining iron(II) and are similar to those found for the trigonal pyramidal, low-spin iron(II) in the series of complexes $[M\{Fe(NS_3)(CO)\text{-}S,S'\}_2]$ (M = Fe, Co, Ni).[19] Due to its insolubility we have been unable to characterise this material further and cannot eliminate the possibility that the material is polymeric. A similar approach, utilising the lability of monophosphines on nickel(II), has been independently developed by Sellmann *et al.*[22] They have reacted the dinuclear iron complex $[Fe(\text{'}S_3\text{'})(CO)_2]_2$ ($\text{'}S_3\text{'}^{2-}$ = bis(mercaptophenyl)-sulfide(2−)) with $[Ni(PMe_3)_2(S_2C_6H_4)]$ to give, in low yield, the crystallographically characterised complex $[Fe(CO)(PMe_3)_2(\mu\text{-'}S_3\text{'})Ni(S_2C_6H_4)]$. In this complex nickel sits in an all-sulfur, square planar coordination environment. The Ni ··· Fe distance of 3.323(1) Å is very close to that found for complexes **1** and **2** (3.308(2) and 3.0216(5) Å, respectively).

An alternative route to NiFe-complexes with nickel in an all-sulfur environment is to react the iron-carbonyl chelate, $[Fe(NS_3)(CO)]^-$, with a nickel dithiocarbamate possessing labile co-ligands, $[NiCl(PPh_3)(S_2CN{}^iPr_2)]$. The product has an elemental analysis consistent with the formulation $[\{Fe(NS_3)(CO)\text{-}S,S'\}Ni(S_2CN{}^iPr_2)]$ **5**, the labile chloride and phosphine ligands again being lost from the nickel. Infra-red peaks, in addition to the carbonyl stretch at 1935 cm^{-1}, are observed at 800, 1022 (characteristic of a delocalised C–S bond in dithiocarbamates)[23] and 1099 cm^{-1}; these bands are also present in the nickel-dithiocarbamate precursor (802, 1020, 1099 cm^{-1}). Absent from the infra-red spectrum of **5** is a peak at 1504 cm^{-1}, characteristic of a dithiosemicarbazone and associated with delocalisation of an electron over the C–N bond, consistent with this electron in **5** residing between the carbon, two sulfur and nickel atoms. The Mössbauer parameters of **5** are consistent with the iron atom remaining low-spin iron(II). Unfortunately, the insolubility again precludes further spectroscopic characterisation or crystal growth for structural analysis.

An iron–nickel-methyl assembly relevant to ACS

The mechanism for the synthesis of acetyl-CoA by the enzyme ACS is yet to be defined unequivocally.[8,9,12,13] However, metal carbonyl, methyl-nickel and acyl-metal intermediates are still implicated.

Reaction of the iron-nitrosyl chelate, $[Fe(NS_3)(NO)]^-$, with $[NiCl(CH_3)(dppe)]$ in acetonitrile gave, in good yield, the methylated-nickel dinuclear complex [{Fe(*N*S$_3$)(NO)-*S*}Ni(CH$_3$)(dppe)] **6** in which the iron moiety is linked to nickel through a single bridging thiolate.‡ Compound **6** is insoluble in most solvents but will dissolve in *N*-dimethylformamide. The structure of **6** is shown in Fig. 5 and selected bond lengths and angles are listed in Table 1. Examination of the crystal structure of **6·0.2MeCN** shows that the nickel and methyl carbon are displaced 0.004(3) and 0.107(13) Å respectively from the SP$_2$ plane, whilst the trigonal bipyramidal iron is displaced 0.242(2) Å towards the apical nitrosyl from the plane of three sulfur ligands. The S$_3$ and SP$_2$ planes are twisted with respect to each other about S(1) with an angle between their normals of 74.82(9)°. The angle about the bridging sulfur, 101.62(12)°, is similar to that in other monothiolate bridged *N*S$_3$ complexes,[17] and substantially greater than the mean 80.59° in the dithiolate bridged **2**. As expected in **6** there is a concomitant increase in the Ni ··· Fe distance to 3.596(2) Å compared with 3.0216(5) Å in **2**. In contrast to [NEt$_4$][Fe(*N*S$_3$)(NO)][17] and compound **2**[19] no disorder is observed in the *N*-methylene groups of the *N*S$_3$ ligand in **6**.

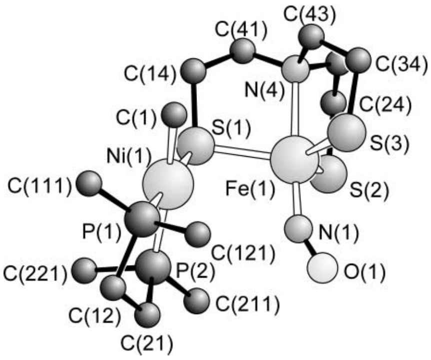

Fig. 5 A molecule of [{Fe(*N*S$_3$)(NO)-*S*}Ni(CH$_3$)(dppe)] **6** showing principal atoms. The phenyl groups of the dppe ligand and all hydrogen atoms have been omitted for clarity.

The nitrosyl ligand is bent at 152.5(9)° about the nitrogen, consistent with NO$^-$, which compares favourably with the mean angle of 147.9° in **2**, as does the nitrosyl infra-red stretch for **6** at 1666 cm^{-1} and for **2** at 1667 cm^{-1}. These values are similar to those of other Fe(*N*S$_3$)-nitrosyl complexes.[17] This limited difference suggests that the change from one bridging thiolate to two bridging thiolates has little effect upon the nitrosyl environment and, from Mössbauer parameters, there is also little effect on the iron environment (**6**, isomer shift (i.s.) 0.40, quadrupole splitting (q.s.) 1.26; **2**, i.s. 0.40, q.s. 1.19 mms^{-1}). These values, and the temperature independent q.s. between 77–298 K, correspond to a formal high-spin iron(III) oxidation state. The Mössbauer parameters are in line with those of other reported five-coordinate high-spin iron(III) complexes, *e.g.* [Fe(salen)Cl]·2MeNO$_2$ (i.s. = 0.40, q.s. = 1.33 mms^{-1}, 80 K).[24] The NO$^-$ ligand ($S = 1$) enforces spin pairing to give an S = 3/2 state arising from antiferromagnetic coupling with a high-spin ferric state (S = 5/2)[25] giving an {FeNO}^{7} complex under the Enemark and Feltham notation.[26]

The ^{1}H-NMR signal for Ni-methyl ($\delta_H = 1.3$) is in accord with those of [Ni(CH$_3$)$_2$(dppe)] (δ_H = 0.8)[27] and [NiCl(CH$_3$)(dppe)] (δ_H = 0.1).[28] The ^{1}NMR shifts for the ethylene (δ_H = 2.2) and phenyl protons (δ_H = 7.3–8.0 ppm) of dppe are the same in [NiCl(CH$_3$)(dppe)], dithiolate-bridged **2** and **6**. Complexes **2** and **6** exhibit ^{31}P NMR signals (referenced to TMP) at −113 and −112 ppm, respectively, which are upfield from dppe (δ_P = −150) and downfield from those of [NiCl(CH$_3$)(dppe)] (δ_P = −79 and −102).

Table 1 Selected molecular dimensions (bond lengths in Å, angles and torsion angles in °) in complex **6**

(a) Bond lengths

Ni(1)–C(1)	1.969(10)	Fe(1)–N(1)	1.754(10)
Ni(1)–S(1)	2.206(3)	S(1)–Fe(1)	2.429(3)
Ni(1)–P(1)	2.120(3)	Fe(1)–S(2)	2.284(3)
Ni(1)–P(2)	2.243(3)	Fe(1)–S(3)	2.286(3)
Ni(1)–Fe(1)	3.596(2)	Fe(1)–N(4)	2.265(8)

(b) Bond angles

C(1)–Ni(1)–S(1)	93.3(4)	N(1)–Fe(1)–S(1)	101.5(3)
C(1)–Ni(1)–P(1)	85.4(4)	N(1)–Fe(1)–S(3)	91.2(3)
C(1)–Ni(1)–P(2)	171.7(4)	N(1)–Fe(1)–S(2)	95.9(3)
P(1)–Ni(1)–S(1)	178.68(13)	N(1)–Fe(1)–N(4)	175.4(4)
S(1)–Ni(1)–P(2)	94.41(12)	S(2)–Fe(1)–S(1)	109.26(12)
P(1)–Ni(1)–P(2)	86.89(12)	S(3)–Fe(1)–S(1)	122.71(13)
		N(4)–Fe(1)–S(1)	82.2(2)
		S(2)–Fe(1)–S(3)	124.78(14)
		N(4)–Fe(1)–S(2)	85.3(2)
		N(4)–Fe(1)–S(3)	84.5(2)
Ni(1)–S(1)–Fe(1)	101.62(12)		
C(14)–S(1)–Ni(1)	117.0(4)		
C(14)–S(1)–Fe(1)	99.8(4)		
C(24)–S(2)–Fe(1)	101.2(4)		
C(34)–S(3)–Fe(1)	101.8(4)		
O(1)–N(1)–Fe(1)	152.5(9)		

(c) Torsion angles

Ni(1)–P(1)–C(12)–C(21)	−38.2(8)
P(1)–C(12)–C(21)–P(2)	50.2(9)
C(12)–C(21)–P(2)–Ni(1)	−42.3(8)
Ni(1)–S(1)–C(14)–C(41)	−145.5(7)
Fe(1)–S(1)–C(14)–C(41)	−37.1(8)
S(1)–C(14)–C(41)–N(4)	57.3(11)
Fe(1)–N(4)–C(41)–C(14)	−46.0(11)
Fe(1)–S(2)–C(24)–C(42)	−23.7(9)
S(2)–C(24)–C(42)–N(4)	50.6(13)
Fe(1)–N(4)–C(42)–C(24)	−49.6(11)
Fe(1)–S(3)–C(34)–C(43)	−29.6(8)
S(3)–C(34)–C(43)–N(4)	50.1(12)
Fe(1)–N(4)–C(43)–C(34)	−43.2(11)

(d) Mean planes

Atoms in plane 1: S(1), S(2), S(3)
Distance from plane 1 to Fe(1): 0.242(2) displaced towards the NO ligand
Atoms in plane 2: S(1), P(1), P(2)
Distance from plane 2 to Ni(1): 0.004(3)
Distance from plane 2 to C(1): 0.107(13)
Angles between the normals to planes 1 and 2: 74.82(9)

Complex **6** is a first generation analogue of a proposed nickel-methyl intermediate in the enzymatic cycle of ACS.[9,13,14] It is the first example of a methylated nickel in a hetero-dimetallic assembly. Mononuclear nickel-methyl complexes such as [NMe$_4$][Ni(CH$_3$)('Me*S$_2$*')$_2$] (where 'Me*S$_2$*'$^-$ = *o*-(methylthio)-thiophenolate(1−))[29] and [Ni(N(CH$_2$CH$_2$SR)$_3$)(CH$_3$)] (where R = ^{i}Pr, ^{t}Bu)[30] can be reacted with carbon monoxide to form nickel-acetyl species whose occurrence can be monitored through the formation of characteristic acetyl bands in the infra-red spectrum. Complex **6** has low solubility and under our conditions does not react with carbon monoxide. We are currently investigating routes to related, more soluble compounds, which are expected to be more reactive.

Experimental

General

All manipulations were performed under a dinitrogen atmosphere, unless otherwise stated, using either Schlenk or vacuum line techniques. Solvents were dried over appropriate drying agents and distilled under dinitrogen prior to use. [NEt$_4$][Fe(*N*S$_3$)(CO)],[16] [NEt$_4$][Fe(NO)(*N*S$_3$)],[17] [NiCl$_2$(PPh$_3$)$_2$][31] and

---

‡ Previously, in the absence of a crystal structure, we proposed a dithiolate-bridge between the nickel and iron.[19]

[NiCl$_2$(dppe)][32] were prepared by published procedures. [NiCl(PPh$_3$)(S$_2$CN^{i}Pr$_2$)] was prepared by adaptation of the synthesis of [NiCl(PPh$_3$){S$_2$CN(Bz^{i}Pr)$_2$}] (where Bz^{i}Pr = benzylisopropyl).[33] An improved synthesis of [NiCl(CH$_3$)(dppe)][28] is described below. IR spectra were recorded on Shimadzu FTIR-8000 spectrophotometer. ^{1}H and ^{31}P NMR were obtained on a Jeol Lambda 400 spectrometer. Mössbauer spectra were recorded on an ES-Technology MS-105 spectrometer with a 925 MBq ^{57}Co source in a rhodium matrix at ambient temperature. Spectra were referenced to a 25 µm iron foil at 298 K and fitted with Lorentzian lines. Elemental analyses were by Mr A. W. L. Saunders at the University of East Anglia (CHN), or by Southern Science, Falmer Laboratories, East Sussex (metals).

Preparations

[Ni{Fe(*N*S$_3$)(CO)-*S,S′*}$_2$] 3. To a solution of [NEt$_4$][Fe(*N*S$_3$)(CO)] (0.59 g, 1.44 mmol) in MeCN (25 cm^3) was added a solution of [NiCl$_2$(PPh$_3$)$_2$] (1.17 g, 1.79 mmol) in MeCN (100 cm^3). The dark red solution that immediately formed was stirred for 90 min under an atmosphere of CO, with no signs of CO uptake. Upon standing overnight, dark crystals formed which were collected by filtration, washed with diethyl ether and dried (0.25 g, 56%). This material showed identical infra-red and Mössbauer spectra to those reported previously.[19,20] The retained filtrate, over a period of three days under a dinitrogen atmosphere, slowly changed to a dark blue colour whilst giving a crystalline precipitate of PPh$_3$. The solution was reduced to a volume of ~15 cm^3 *in vacuo* and filtered to remove PPh$_3$. Diethyl ether (5 cm^3) was added to the blue filtrate leading to the formation of large royal blue crystals of [NEt$_4$][NiCl$_3$(PPh$_3$)] and white crystals of [NEt$_4$]Cl. These were collected by filtration, washed with diethyl ether and dried *in vacuo*.

[{Fe(*N*S$_3$)(CO)-*S,S′*}Ni(SCH$_2$CH$_2$S)] 4. Under an atmosphere of carbon monoxide, a solution of [NiCl$_2$(PPh$_3$)$_2$] (0.79 g, 1.50 mmol) in MeCN (80 cm^3) containing ethanedithiol (0.10 cm^3, 1.19 mmol) was added to a solution of [NEt$_4$][Fe(*N*S$_3$)(CO)] (0.49 g, 1.20 mmol) in MeCN (30 cm^3). The solution immediately turned a dark red colour associated with the formation of [Ni{Fe(*N*S$_3$)(CO)-*S,S′*}$_2$] but quickly turned brown upon stirring. No CO gas uptake was observed. After stirring for two hours the reaction mixture was left to stand overnight. A dark brown solid was collected by filtration, washed repeatedly with diethyl ether and dried (0.23 g, 45.0%) (Found: C, 25.3; H, 3.7; N, 2.8; Fe, 13.2; Ni, 14.7. C$_9$H$_{16}$FeNNiOS$_5$ requires: C, 25.2; H, 3.8; N, 3.3; Fe, 13.0; Ni, 13.7%); ν_{max}/cm^{-1} (CO) 1935 (KBr); Mössbauer, i.s. = 0.20, q.s. = 1.34 mms, $\Gamma_{½}$ = 0.17 mms^{-1}.

[{Fe(*N*S$_3$)(CO)-*S,S′*}Ni(S$_2$CN^{i}Pr$_2$)] 5. [NiCl(PPh$_3$)(S$_2$CN^{i}Pr$_2$)] (0.14 g, 0.26 mmol) dissolved in MeCN (30 cm^3) was layered by syringe on to the surface of a solution of [NEt$_4$][Fe(*N*S$_3$)(CO)] (0.11 g, 0.27 mmol) in MeCN (20 cm^3). Overnight, crystals of [Fe{Fe(*N*S$_3$)(CO)-*S,S′*}$_2$] (<0.01 g) formed and were removed by filtration. Stirring the filtrate again overnight gave a dark brown solid that was collected by filtration, washed repeatedly with MeCN and then diethyl ether before being dried. (0.03 g, 22.3%) (Found: C, 32.1; H, 5.3; N, 5.7; Fe, 10.2; Ni, 10.7. C$_9$H$_{16}$FeNNiOS$_5$ requires: C, 32.8; H, 4.9; N, 5.5; Fe, 10.9; Ni, 11.5%); ν_{max}/cm^{-1} (CO) 1935 (KBr); Mössbauer, i.s. = 0.21, q.s. = 1.16 mms, $\Gamma_{½}$ = 0.20 mms^{-1}.

[NiCl(CH$_3$)(dppe)]. MeMgI (12 cm^3 of a 3.0 M diethyl ether solution, 36 mmol) was added to [NiCl$_2$(dppe)] (1.42 g, 2.69 mmol) in tetrahydrofuran (65 cm^3). After stirring for 2 days the solution was concentrated to ~ 40 cm^3 and filtered through approx. 1 cm of Celite. Ethanol (5 cm^3) was added to the filtrate which was then refiltered. The filtrate volume was halved under reduced pressure and ethanol (100 cm^3) added. Honey-brown rhombic crystals precipitated over two days at −20 °C, these were collected by filtration and dried (0.45g, 33.0%) (Found: C, 64.1; H, 5.6. Expected for C$_{27}$H$_{27}$ClNiP$_2$ requires: C, 63.9; H, 5.4%); δ_H(Me) (400 MHz, dmf-d$_7$, standard SiMe$_4$) 0.1.

[{Fe(*N*S$_3$)(NO)-*S*}Ni(CH$_3$)(dppe)]·0.2MeCN 6·0.2MeCN. A solution of [NiCl(CH$_3$)(dppe)] (0.13 g, 0.26 mmol) in MeCN (40 cm^3) was layered onto the surface of a solution of [NEt$_4$][Fe(NO)(*N*S$_3$)] (0.11 g, 0.23 mmol) in MeCN (20 cm^3). Black square plates formed after two days that were collected by filtration, washed repeatedly with diethyl ether and then dried (0.14 g, 69%) (Found: C, 52.2; H, 5.2; N, 4.5; Fe, 6.9; Ni, 7.7. C$_{33}$H$_{39}$FeN$_2$NiOP$_2$S$_3$.C$_{0.2}$H$_{0.6}$N$_{0.2}$ requires: C, 52.7; H, 5.2; N, 4.1; Fe, 7.3; Ni, 7.7%); λ_{max}/nm (dmf) 265 (ε/dm^3 mol^{-1} cm^{-1} 30053), 318 (9309), 375 (5053), 464 (2394); ν_{max}/cm^{-1} (NO) 1666 (KBr); δ_H(Me) (400 MHz; dmf-d$_7$; standard SiMe$_4$) 1.3; δ_P(162 MHz; dmf-d$_7$; standard P(OMe)$_3$) −112; Mössbauer, i.s. = 0.40, q.s. = 1.19, $\Gamma_{½}$ = 0.17 mms^{-1}.

Crystal structure analysis of complex 6

Crystals of [{Fe(NO)(*N*S$_3$)-*S*}Ni(CH$_3$)dppe]·0.2MeCN were black square plates. A single crystal of size 0.24 × 0.17 × 0.06 mm (corresponding to the *c, a* and *b* axes of the unit cell) was selected and mounted on a glass fibre for photographic examination and the data collection. Diffraction data were measured on an Nonius CAD4 diffractometer with monochromated Mo-Kα radiation. The data were collected at 140 K, with the temperature being lowered slowly to avoid shocking the crystal. Cell parameters were refined using least-squares methods from the settings of 24 reflections in the range 10 to 11° in θ centred in 4 orientations. 4173 Unique reflections were collected to θ of 22° the limit of observable intensities, with 1854 having intensities greater than $2\sigma_I$. There were no change in orientations or decay of intensities during data collection.

The data were corrected for Lorentz-polarisation effects, for absorption by semi-empirical psi-scan methods, and for negative intensities by Bayesian statistical methods. The structure was solved using direct methods in the SHELXS-86 program[34] and refined on F^2 using full-matrix least-squares procedures in SHELXL-97.[35] All non-hydrogen atoms were refined with anisotropic displacement parameters, except some unresolved solvent atoms, which were all refined isotropically as carbon atoms. Hydrogen coordinates were of idealised positions and set to ride on the parent atoms in the complex molecule, with thermal displacement parameters refined isotropically. Convergence was reached with $R_1 = 0.148$ and $wR_2 = 0.138$ for all data weighted $w = 1/[\sigma^2(F_o^2) + (0.013P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$; for the 1854 observed data $R_1 = 0.067$ and $wR_2 = 0.100$.

In the final difference map the residual electron density was 0.52 e$^-$ Å^{-3} for the largest peak, which lay between the Ni and the methyl carbon. Scattering factors were taken from ref. 36. Computer programs used in the analysis have been noted above and/or in Table 4 of ref. 37, and were run on a DEC-AlphaStation 200 4/100 in the Department of Biological Chemistry, John Innes Centre.

Crystal data: C$_{33}$H$_{39}$FeN$_2$NiOP$_2$S$_3$,0.2(C$_2$H$_3$N), M = 760.5, monoclinic, space group $P2_1/c$ (no. 14), a = 11.410(3), b = 19.361(3), c = 15.633(2) Å, α = 90°, β = 98.64(2)°, γ = 90°, V = 3414.3(17) Å^3. Z = 4, D_c = 1.480 g cm^{-3}, F(000) = 1582, T = 140(1) K, μ(Mo-Kα) = 1.284 mm^{-1}, λ(Mo-Kα) = 0.71069 Å.

CCDC reference number 192738.

See http://www.rsc.org/suppdata/dt/b3/b307175h/ for crystallographic data in CIF or other electronic format.

Acknowledgements

The Biotechnology and Biological Sciences Research Council are thanked for funding. Dr J. R. Sanders of the Dept. of

Biological Chemistry, John Innes Centre is thanked for the supply of NS_3H_3.

References

1 Y. Nicolet, C. Piras, P. LeGrand, C. E. Hatchikian and J. C. Fontecilla-Camps, *Structure*, 1997, **7**, 13.

2 J. W. Peters, W. N. Lanzilotta, B. J. Lemon and L. C. Seefeldt, *Science*, 1998, **282**, 1853.

3 A. J. Pierik, M. Hulstein, W. R. Hagen and S. P. J. Albracht, *Eur. J. Biochem.*, 1998, **258**, 572; A. L. De Lacey, C. Stadler, C. Cavazza, E. C. Hatchikian and V. M. Fernandez, *J. Am. Chem. Soc.*, 2000, **122**, 11232; Y. Nicolet, A. L. De Lacey, X. Vernede, V. M. Fernandez, E. C. Hatchikian and J. C. Fontecilla-Camps, *J. Am. Chem. Soc.*, 2001, **123**, 1596.

4 M. Razavet, S. C. Davies, D. L. Hughes, J. E. Barclay, D. J. Evans, S. A. Fairhurst, X. Liu and C. J. Pickett, *J. Chem. Soc., Dalton Trans.*, 2003, 586.

5 A. Volbeda, M.-H. Charon, C. Piras, E. C. Hatchikian, M. Frey and J. C. Fontecilla-Camps, *Nature*, 1995, **373**, 580; A. Volbeda, E. Garcin, C. Piras, A. L. de Lacey, V. M. Fernandez, E. C. Hatchikian, M. Frey and J. C. Fontecilla-Camps, *J. Am. Chem. Soc.*, 1996, **118**, 12989.

6 R. P. Happe, W. Roseboom, A. Pierik, S. P. J. Albracht and K. A. Bagley, *Nature*, 1997, **385**, 126.

7 P. M. Matias, C. M. Soares, L. M. Saraiva, R. Coelho, J. Morais, J. Le Gall and M. A. Carrondo, *J. Biol. Inorg. Chem.*, 2001, **6**, 63 and references therein.

8 T. I. Doukov, T. M. Iverson, J. Seravalli, S. W. Ragsdale and C. L. Drennan, *Science*, 2002, **298**, 567.

9 C. Darnault, A. Volbeda, E. J. Kim, P. Legrand, X. Vernède, P. A. Lindahl and J. C. Fontecilla-Camps, *Nat. Struct. Biol.*, 2003, **10**, 271.

10 H. Dobbek, V. Svetlitchnyi, L. Gremer, R. Huber and O. Meyer, *Science*, 2001, **293**, 1281.

11 C. L. Drennan, J. Heo, M. D. Sintchak, E. Schreiter and P. Ludden, *Proc. Nat. Acad. Sci. USA*, 2001, **98**, 11973.

12 J. Seravalli, W.-W. Gu, A. Tam, E. Strauss, T. P. Begley, S. P. Cramer and S. W. Ragsdale, *Proc. Nat. Acad. Sci. USA*, 2003, **100**, 3689.

13 S. Gencic and D. A. Grahame, *J. Biol. Chem.*, 2003, **278**, 6101.

14 J. C. Fontecilla-Camps and S. W. Ragsdale, *Adv. Inorg. Chem.*, 1999, **47**, 283 and references therein.

15 C. R. Staples, J. Heo, N. J. Spangler, R. L. Kerby, G. P. Roberts and P. W. Ludden, *J. Am. Chem. Soc.*, 1999, **121**, 11034; J. Heo, C. R. Staples, C. M. Halbleib and P. W. Ludden, *Biochemistry*, 2000, **39**, 7956.

16 S. C. Davies, D. L. Hughes, M. C. Durrant, R. L. Richards and J. R. Sanders, *J. Chem. Soc., Dalton Trans.*, 2000, 4694.

17 S. C. Davies, D. J. Evans, D. L. Hughes, M. Konkol, R. L. Richards, J. R. Sanders and P. Sobota, *J. Chem. Soc., Dalton Trans.*, 2002, 2473.

18 S. C. Davies, D. J. Evans, D. L. Hughes, S. Longhurst and J. R. Sanders, *Chem. Commun.*, 1999, 1935.

19 M. C. Smith, J. E. Barclay, S. P. Cramer, S. C. Davies, W.-W. Gu, D. L. Hughes, S. Longhurst and D. J. Evans, *J. Chem. Soc., Dalton Trans.*, 2002, 2641.

20 M. C. Smith, S. Longhurst, J. E. Barclay, S. P. Cramer, S. C. Davies, D. L. Hughes, W.-W. Gu and D. J. Evans, *J. Chem. Soc., Dalton Trans.*, 2001, 1387.

21 M. C. Smith, S. C. Davies, D. L. Hughes and D. J. Evans, *Acta Crystallogr., Sect. E.*, 2001, **57**, m509.

22 D. Sellmann, F. Geipel, F. Lauderbach and F. W. Heinemann, *Angew. Chem. Int. Ed.*, 2002, **41**, 632.

23 R. Pastorek, Z. Travnicek, Z. Sindelar and F. Brezina, *Polyhedron*, 1996, **15**, 3691.

24 G. M. Bancroft, A. G. Maddock and R. P. Randl, *J. Chem. Soc. A*, 1968, 2939.

25 C. A. Brown, M. A. Pavlovsky, T. E. Westre, Y. Zhang, B. Hedman, K. O. Hodgson and E. I. Solomon, *J. Am. Chem. Soc.*, 1995, **117**, 715; C. Hauser, T. Glaser, E. Bill, T. Weyhermüller and K. Wieghardt, *J. Am. Chem. Soc.*, 2000, **122**, 4352 and references therein.

26 J. H. Enemark and R. D. Feltham, *Coord. Chem. Rev.*, 1974, **13**, 339.

27 M. L. H. Green and M. J. Smith, *J. Chem. Soc. A*, 1971, 639.

28 T. Yamamoto, T. Kohara, K. Osakada and A. Yamamoto, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2147.

29 D. Sellmann, H. Schillinger, F. Knoch and M. Moll, *Inorg. Chim. Acta*, 1992, **200**, 351.

30 P. Stavropoulos, M. C. Muetterties, M. Carrie and R. H. Holm, *J. Am. Chem. Soc.*, 1991, **113**, 8485.

31 R. E. King, S. B. Miller, C. B. Knobler and M. F. Hawthorne, *Inorg. Chem.*, 1983, **22**, 3548.

32 S. C. Davies, R. A. Henderson, D. L. Hughes and K. E. Oglieve, *J. Chem. Soc., Dalton Trans.*, 1998, 425.

33 R. Pastorek, J. Kamenicek, Z. Travnicek, J. Husarek and N. Duffy, *Polyhedron*, 1999, **18**, 2879.

34 G. M. Sheldrick, *Acta Crystallogr., Sect. A*, 1990, **46**, 467.

35 G. M. Sheldrick, SHELXL-97, Program for refinement of crystal structures, University of Göttingen, Germany, 1997.

36 *International Tables for X-ray Crystallography*, Kluwer Academic Publishers, Dordrecht, 1992, vol. C, pp. 500, 219 and 193.

37 S. N. Anderson, R. L. Richards and D. L. Hughes, *J. Chem. Soc., Dalton Trans.*, 1986, 245.