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Metal–sulfur chemistry relevant to modelling the active sites of some enzymes of environmental importance

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Recent advances in the synthesis of metal-sulfur complexes may lead to the development of alternative catalysts for use in fuel cell technology.

Keywords: metal-sulfur chemistry, fuel-cell technology

Introduction

Hydrogen is a key feedstock in the chemical and petrochemical industries and is recognised as an important fuel for the future. Fuel cell technology, associated with the conversion of hydrogen to energy, is dependent on precious metal catalysts. The water-gas shift reaction (WGSR) and the Monsanto modification of the Reppe process (MRP) are extremely important to industry. The WGSR utilises the reducing power of carbon monoxide to generate carbon dioxide and hydrogen from water at high temperature, eqn (1). The MRP synthesises acetic acid from methanol and carbon monoxide using a rhodium based catalyst at 180°C and 30 bar, eqn (2). In contrast, the hydrogenase

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (1)$$

$$CH_3OH + CO \rightarrow CH_3COOH \quad (2)$$

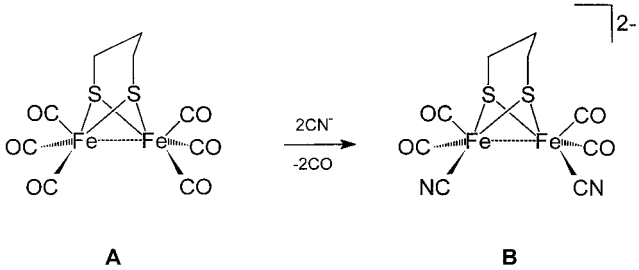
enzymes and nickel CO-dehydrogenase/acetyl-CoA synthase (CODH/ACS) enzymes can perform the equivalent conversions, under physiological conditions, with active sites consisting of the metals nickel and iron. The hydrogenases catalyse the reversible conversion of dihydrogen to protons and electrons. The CODH reaction is formally equivalent to the WGSR except it produces two protons and two electrons rather than dihydrogen. 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 MRP, both it seems involving metal carbonyl, methyl-metal and acyl-metal intermediates. In addition, through the coupled reactions of CODH/ACS, carbon dioxide and highly toxic carbon monoxide are not only removed from the environment but are used as a source of cell carbon. These last conversions are of current relevance to the need to reduce carbon oxide emissions from industry and their levels in the environment. Here I will describe the most recent advances in the synthesis of new metal-sulfur complexes related to the structure of the active sites of each class of enzyme. It is anticipated that these studies will inform the development of alternative catalysts inspired by the biological systems. Earlier chemical modelling has been reviewed by others: Fe-only hydrogenase,[1,2] NiFe-hydrogenase,[2,3] CODH/ACS.[4]

Hydrogenases

Iron-only hydrogenase

X-ray crystal structures of Fe-only hydrogenases isolated from *Desulfovibrio desulfuricans*[5] and *Clostridium pasteurianum*,[6] together with FTIR data from *D. desulfuricans*[7] and *D. vulgaris*,[8] show that the active site (the H-centre), at which protons are reduced to dihydrogen, is comprised of an Fe_4S_4-cluster linked by a bridging cysteinyl to an "organometallic" diiron sub-site. The iron atoms of the sub-site are each bound by CN^-, a terminal CO and, in the oxidised state, a bridging CO. On reduction the bridging CO switches to a terminal position.[7] The *exo* iron atom is either coordinatively unsaturated[5] or is ligated with a water molecule.[6] In addition, the two iron atoms are bridged through 1,3-propanedithiolate, Fig. 1, or possibly the related di(thiolatomethyl)amine unit.[7] In the CO inhibited forms of the oxidised enzymes from *C. pasteurianum* and *D. desulfuricans*, it has been shown by crystallography[9] and FTIR spectroscopy,[7] respectively, that an additional molecule of CO is bound at the *exo* iron atom, displacing water or filling the vacant site.

The propanedithiolate-bridged diiron sub-site has precedent in the organometallic diiron hexacarbonyls $[(CO)_3Fe(SC_3H_6S)Fe(CO)_3]$, **A**.[10] Within a year of publication of crystallographic results on the proteins, our laboratory[11], and two other groups,[12, 13] independently reported the reaction of **A** with two equivalents of $[NEt_4]CN$ to give $[NEt_4]_2[(CO)_2(NC)Fe(SC_3H_6S)Fe(CO)_2(CN)]$, B, in which two terminal carbonyls are each replaced by cyanide, Scheme 1. The anion of **B** has clear similarities to the diiron sub-site of the H-cluster. The Fe–Fe distance (2.53 Å) is slightly shorter than in the protein structures (2.6 Å) and all are consistent with the presence of an iron–iron bond. The published X-ray crystal structure of **B** shows the cyanide molecules to be staggered, the position of the CN^- and CO ligands being defined by the difference in the Fe-CX (X = N *vs* O) distances.[12] However, a normal coordinate analysis of the CO FTIR data for our complex **B** shows that the pattern of ν(CO) bands is consistent with the dianion possessing pseudo-C_S symmetry with eclipsed CN^- ligands.[11] The origin of the different assignments is probably that the former salt was prepared at low temperature, giving rise to one isomer, and the latter at room temperature, giving rise to the other isomer. The



A **B**

Scheme 1

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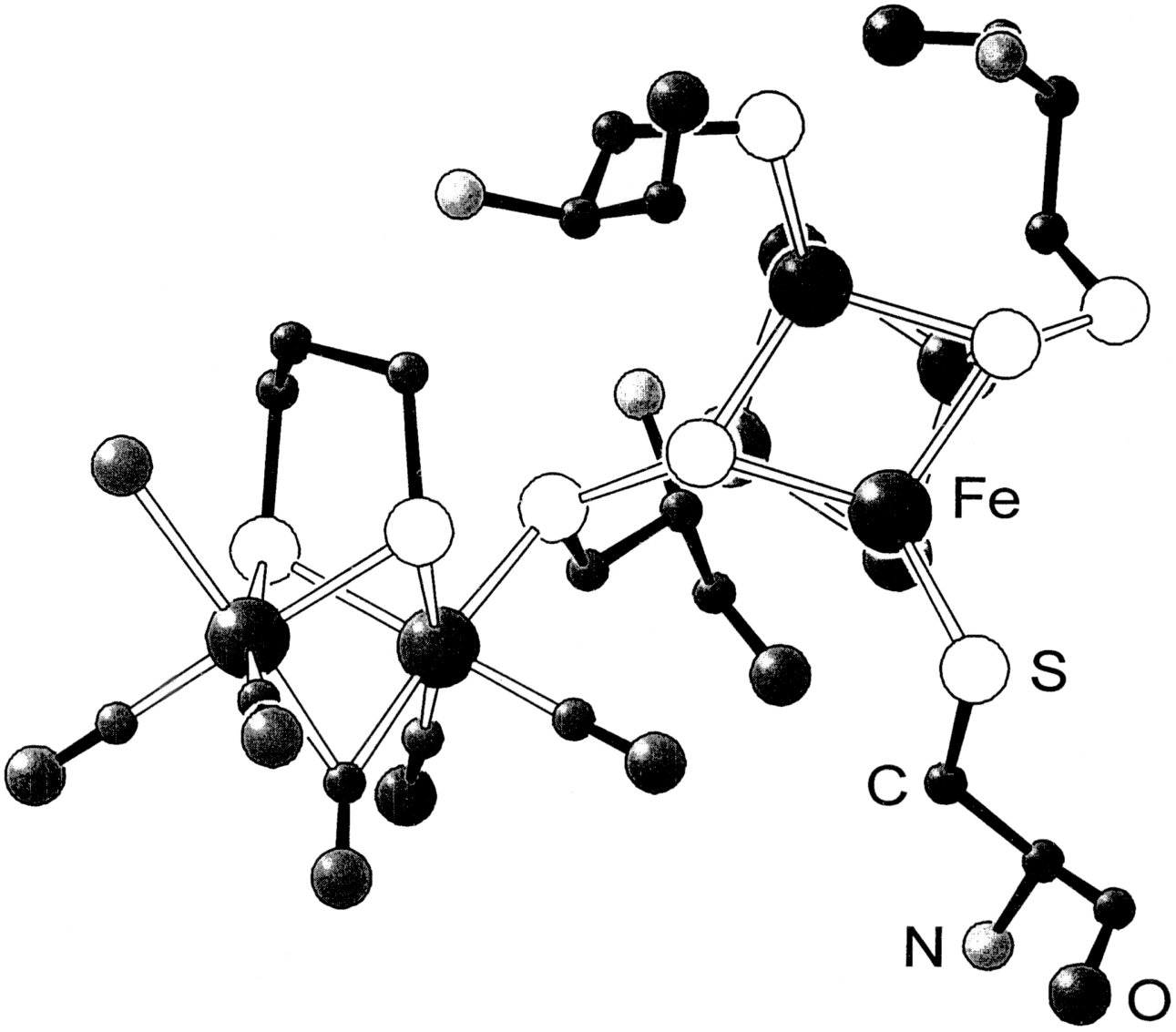


Fig. 1 Proposed composite structure of the H-centre of Fe-only hydrogenase.

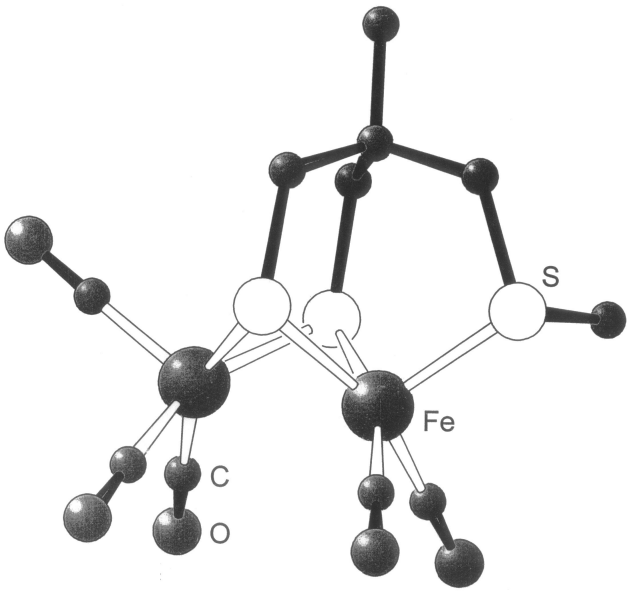


Fig. 2 View of molecule **C** – an Fe_2S_3-cluster related to the diiron sub-site of the H-centre of Fe-only hydrogenase.

eclipsed arrangement of CN^- ligands resembles the arrangement at the diiron sub-site in the CO-inhibited form of the enzyme which possesses a distal $Fe(CO)_2(CN)$ site.[9] The complex **B**, unsurprisingly, as it is analogous to the diiron sub-site of the CO-inhibited enzyme, neither reacts with nor electrocatalyses the reduction of protons in aqueous solution over the pH range 4.0–8.4.[11]

Pickett and his group in our laboratory have now synthesised a closer structural analogue by the use of a tripodal dithiolate thioether ligand which allows the synthesis of a diiron pentacarbonyl with differential (2:3) sulfur ligation of the iron atoms, complex **C**, Fig. 2.[14] The Fe–Fe bond distance and the average bridging Fe–S bond lengths are similar to those distances for the sub-site within the enzyme, but the thioether Fe–S distance in **C** (2.25 Å) is shorter than the corresponding sub-site $Fe–S_{cysteine}$ distance in the enzyme (ca 2.5 Å).[5,6] The reaction of **C** with two equivalents of cyanide, as monitored by FTIR, show that initially a CO-bridged dicyanide species, **D**, is formed which slowly isomerises to the metal-metal bonded terminal carbonyl species with dissociation of the methyl thioether group, Scheme 2. This indicates that a model complex is obtainable with all the principal structural features of the oxidised CO-inhibited form of the natural sub-site: two

cyanide groups, terminal and bridging CO ligands and an Fe_2S_3-core.[14]

Infrared spectroscopy of the Fe^{I}–Fe^{I} model complexes **B** and **C** show ν(CN) and ν(CO) bands similar to those of the reduced enzyme.[8] Well resolved infrared data for the oxidised CO-inhibited form of the H-centre have been reported.[15] The diiron sub-site in this paramagnetic state has been described as a localised mixed-valence state, though whether this was Fe^{II}–Fe^{III} or Fe^{I}–Fe^{II} was undecided.[16] De Lacey *et al.* have deduced from the infrared spectra that the proximal iron atom of the sub-site is Fe^{II} and the less perturbed distal iron atom is Fe^{I}.[15] Comparison with the infrared parameters for complex **D** supports this deduction.[14]

An analogue of the alternative di(thiolatomethyl)amine-bridged diiron sub-site has been prepared also, Scheme 3, in which the N-group is methylated.[17] The next major challenge in the synthesis of a "free-standing" H-centre is the coupling of a diiron complex to an Fe_4S_4-cluster. The reaction of a 3:1 site-differentiated Fe_4S_4-cluster[18] with a complex such as **C**, but with a free thiolate at one iron atom, should facilitate such an assembly.

NiFe-hydrogenase

The NiFe-hydrogenase from *D. gigas*, as aerobically isolated in the inactive form, has been characterised by X-ray crystallography.[19, 20] The active site is a dinuclear thiolate-bridged nickel-iron complex in which the nickel atom is coordinated by four cysteinate-sulfur atoms, two of which bridge to an iron atom. In the aerobically isolated crystals there is an additional bridging feature, probably oxo or hydroxo, which is unlikely to be present in the active form of the enzyme.[20, 21] The other ligands to iron, as shown by crystallography and spectroscopy,[20] are two cyanides and one carbon monoxide, Fig. 3. The crystal structures of the NiFe-hydrogenase from *D. vulgaris* Miyazaki F[22] and *D. desulfuricans* ATCC 27774[23] have also been reported. The active site structures are similar to that found in *D. gigas.*

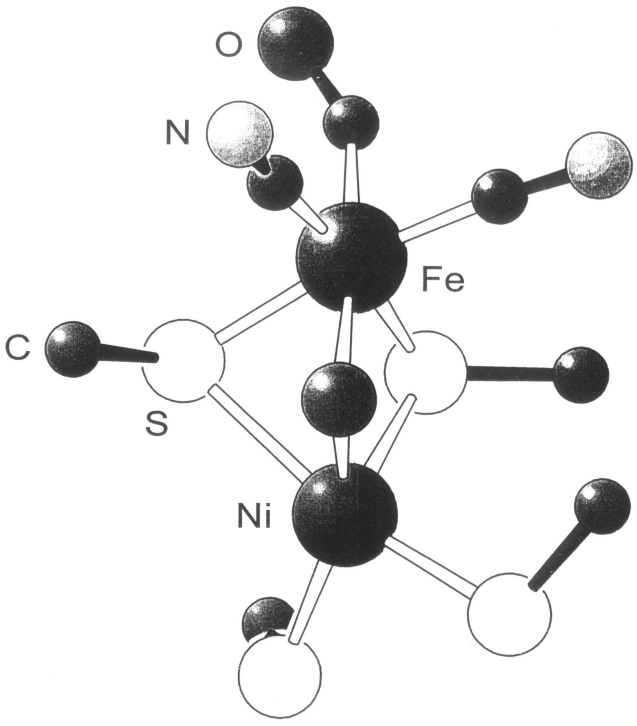
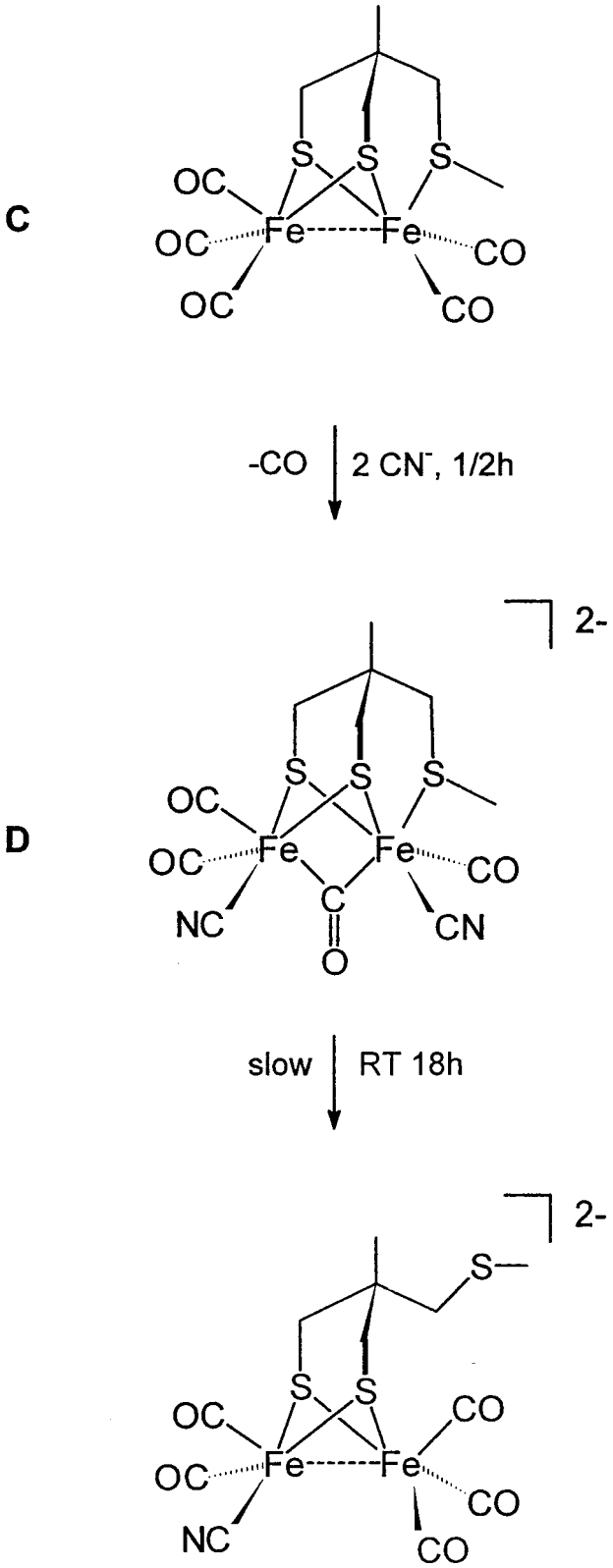


Fig. 3 Proposed structure of the active centre of the aerobically isolated NiFe-hydrogenase from *D. gigas.*

The chemistry of the tripodal tetradentate proligand $N(CH_2CH_2SH)_3$, NS_3H_3, with various transition metals,[24,25] including iron,[25] has been developed by Richards and Sanders. Their complex $[Fe(NS_3)(CO)]^-$ when reacted with



Scheme 2

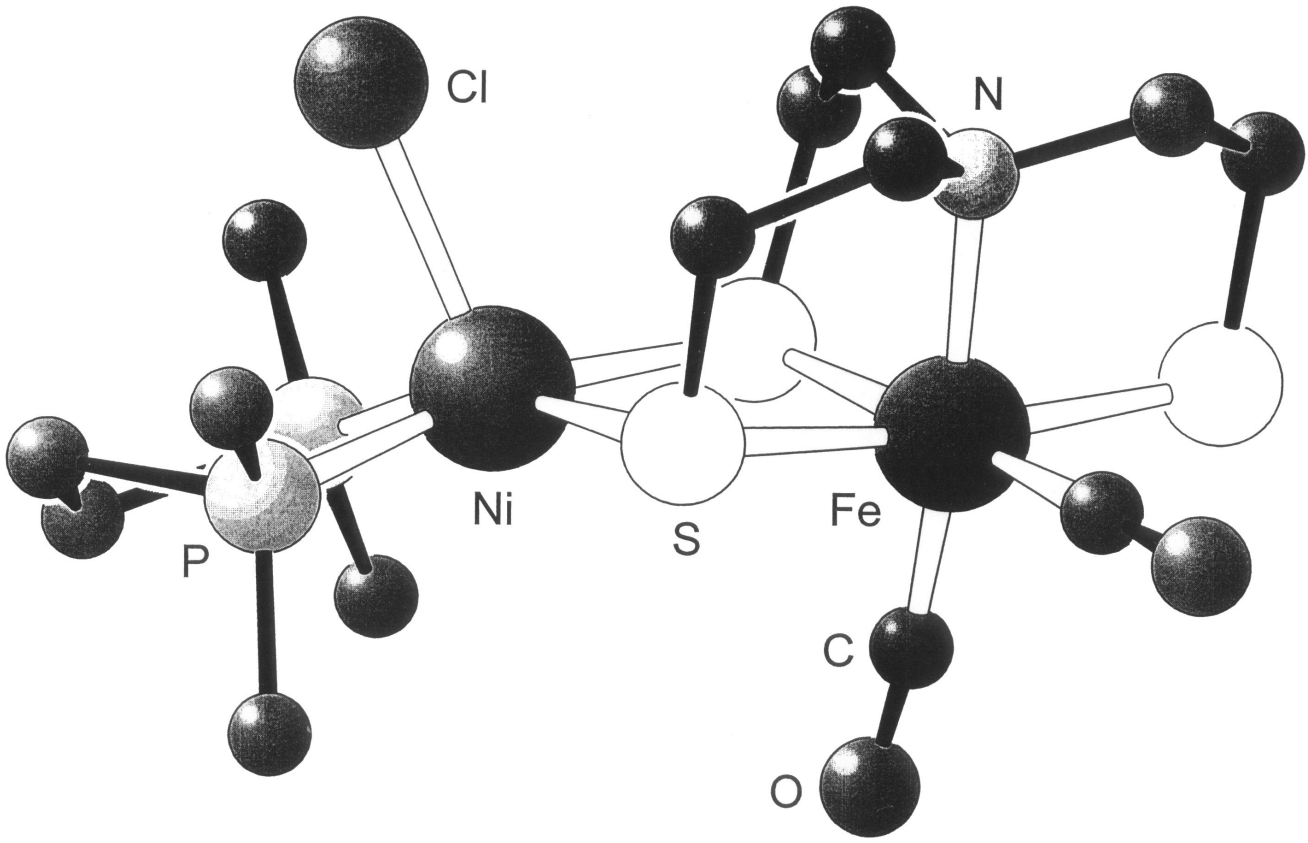
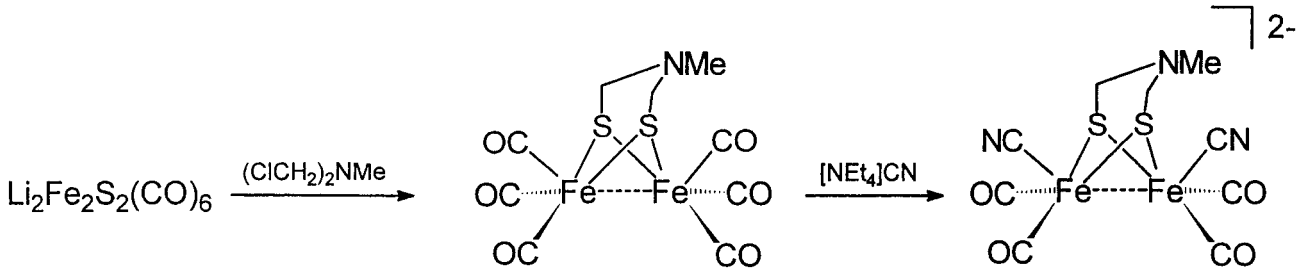


Fig. 4 A molecule of [{Fe(NS_3)$(CO)_2$-S,S=}NiCl(dppe)], **E**, showing the principal atoms. The phenyl groups have been omitted for clarity.



Scheme 3

[$NiCl_2$(dppe)], under an atmosphere of CO, enabled us to prepare a bis(thiolate-bridged) nickel-iron complex, [{Fe(NS_3)$(CO)_2$-*S*,*S*′}NiCl(dppe)], **E**, Fig. 4.[26] Complex **E** is a good structural analogue of the active site of NiFe-hydrogenase, Fig. 5. The core of **E** is dinuclear with nickel bound to iron by a bis(thiolate-bridge) and the iron atom binds two CO's. The Ni···Fe distance of 3.308Å is longer than the Ni···Fe distance, 2.9Å, in the as isolated, inactive NiFe-hydrogenase, [19,20] but similar to the calculated Ni···Fe distance, 3.2Å, for the Ni-Fe unit in the activated enzyme.[27] Prior to this work, there were no examples in the chemical literature of synthetic analogues with bis(thiolate-bridged) nickel to iron with carbon monoxide bound to the iron atom, although [{NiL-*S*}Fe$(CO)_4$] {H_2L = *N*,*N*′-bis(ethanethiol)-1,5-diazacyclooctane}[28] and [{NiL=-*S*,*S*′}Fe$(NO)_2$] (H_2L=*N*,*N*′-diethyl-3,7-diazanonane-1,9-dithiolate)[29] have one or other of these features. The complex **E** does not evolve dihydrogen from a source of protons (ethanoic acid) in the presence of a chemical reductant. However, preliminary results show that the trinuclear $NiFe_2$-complex, [Ni{Fe(NS_3)(CO)-*S*,*S*′}$_2$], **F**, described below, can generate hydrogen but in a less than stoichiometric yield, in a non-catalytic reaction. The analagous trinuclear MFe_2-complexes (M = Fe or Co)[25] do not generate dihydrogen under similar conditions.[30]

A heterobinuclear tris(thiolate-bridged) nickel-iron amine thiolate complex has recently been described. Although this compound is not a good structural model of the active site of the NiFe-hydrogenase, it is the first example of a thiolate-bridged nickel-iron complex which can exist in several oxidation states, *i.e.* $Ni^{II}Fe^{II}$, $Ni^{II}Fe^{III}$ and $Ni^{III}Fe^{III}$.[31]

CO-dehydrogenase/acetyl-CoA synthase

CODH/ACS is usually a bifunctional enzyme performing both CO_2/CO interconversion and carbon fixation in anaerobic microbes.[32] The organism *Rhodospirillum rubrum* shows CODH activity but no ACS activity. There is, at present, no high resolution crystal structure of a CODH/ACS or CODH

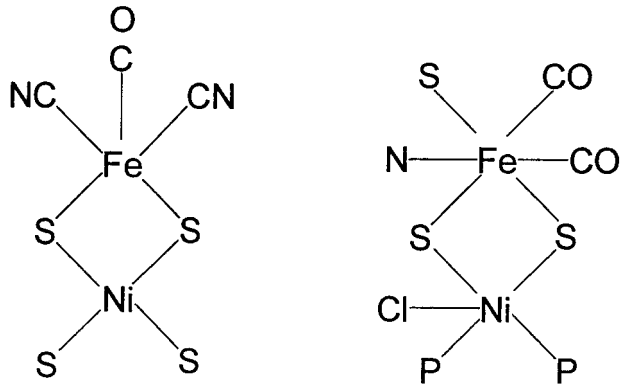


Fig. 5 A schematic comparison of the proposed active site of NiFe-hydrogenase (left) and the core of complex **E** (right).

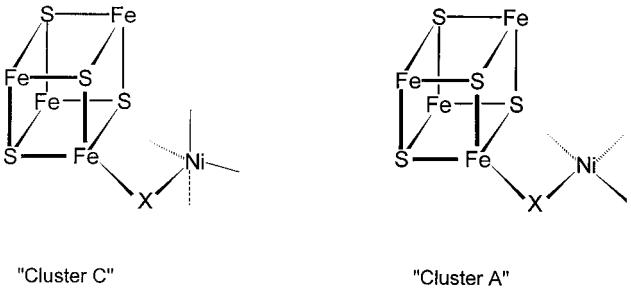
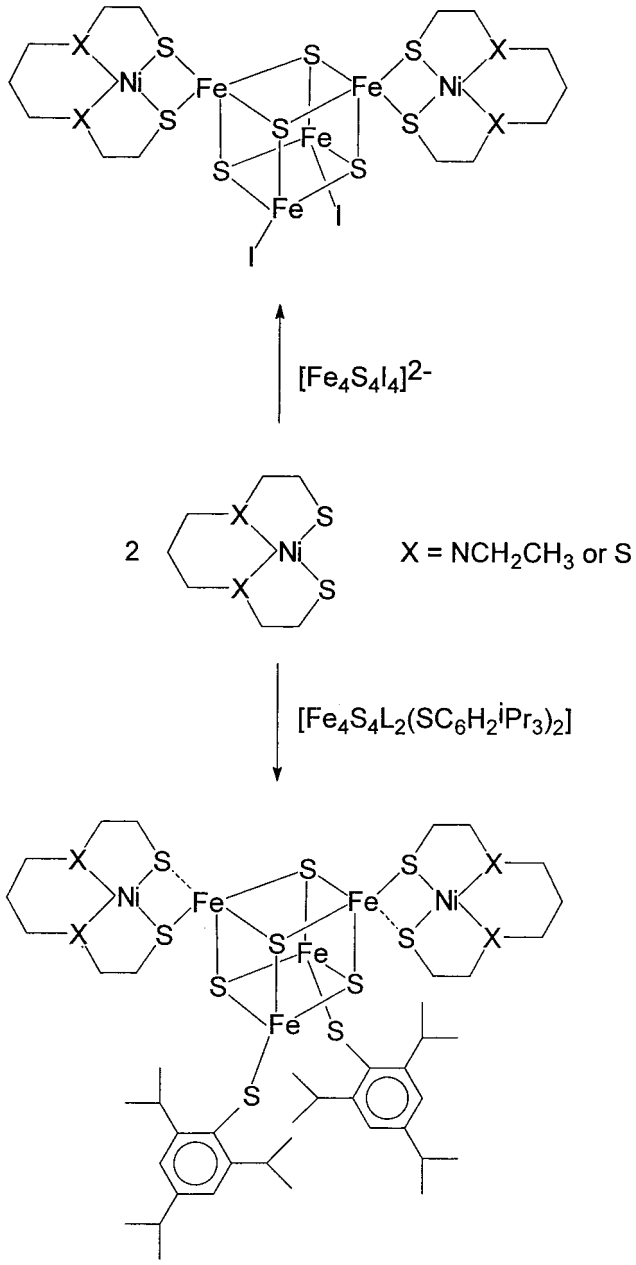


Fig. 6 Proposed structure of the active centres of the bifunctional enzyme CODH/ACS.

protein. However, extensive biochemical and spectroscopic studies have identified two active sites.[32] CODH activity occurs at a centre identified as "Cluster C" and ACS activity at "Cluster A". Until very recently "Cluster C" was described as possessing a five-coordinate or tetrahedrally ligated nickel atom bridged through an unknown atom (probably thiolate or sulfide) to an Fe_4S_4 cluster and "Cluster A" has been proposed to consist of a nickel atom, in square planar geometry, again bridged to an Fe_4S_4 cluster, Fig. 6.

The synthesis of compounds with Ni^{II}-thiolate fragments coupled to Fe_4S_4 clusters has been achieved by Pohl and coworkers.[33] The formation of μ_2-thiolate polynuclear assemblies was avoided by the use of nickel precursor complexes, [*N*,*N*′-diethyl-3,7-diazanonane-1,9-dithiolato]nickel(II) or [3,7-dithianonane-1,9-dithiolato]nickel(II), in which two nickel coordination sites were blocked by non-bridging atoms. The complexes prepared comprised, in the solid state, an $[Fe_4S_4]^{2+}$ unit coupled to two Ni^{II}-complexes either bridged through two or one μ_2-sulfur atoms, dependent upon the other ligands to the Fe_4S_4-cluster, Scheme 4. The coordination geometries and metrical parameters about nickel in the assemblies are in reasonable agreement with those obtained from EXAFS of the enzymes. A preliminary structure has been reported of a related compound in which one nickel complex has been coupled, through two μ_2-sulfur atoms, to an Fe_4S_4–cluster, Fig. 7.[34] The reported crystal structures of these complexes reveal open sites at the μ_2-sulfur bound iron atoms and at the square planar coordinated nickel(II). This observation is consistent with the concept of substrate binding at an analogous centre in the enzymes. In addition, variations of the ligands to nickel and the character of the other ligands to the Fe_4S_4 core changes the constitution of the bridged assemblies. It has been noted[33] that the binding of substrate to, or a change in oxidation level of, the Ni-Fe_4S_4 assemblies in CODH/ACS



Scheme 4

could possibly effect similar structural changes which may be essential to the catalytic properties of the enzymes.

It has since been proposed that the active centre of CODH from *R. rubrum* contains a binuclear FeNi-fragment linked, probably, through cysteinate-sulfur to an Fe_4S_4 cluster. The iron(II) and nickel(II) atoms within the FeNi-fragment are said to be bridged through two cysteinate-sulfurs and, in addition, that there is a nitrogen (from histidine) and a molecule of, non-substrate, CO bound to the iron(II) atom, the other ligands are yet to be identified, Fig. 8.[35] In our laboratory we have prepared the structurally characterised trinuclear linear complex [Ni{Fe(*N*S_3)(CO)-*S*,*S*′}$_2$], **F**, Fig. 9, from reaction of the iron(II)-carbonyl chelate, [Fe(*N*S_3)(CO)]$^-$, with the dimethylsulfoxide solvate of nickel(II) chloride, an assembly which has structural features similar to those proposed for this active site.[36] The common features are, Fig. 8: CO and N coordinated to iron(II); iron bridged through two μ_2-thiolate-sulfurs to nickel; nickel bridged through a further thiolate-sulfur to another iron atom (part of the Fe_4S_4 cluster in the enzyme

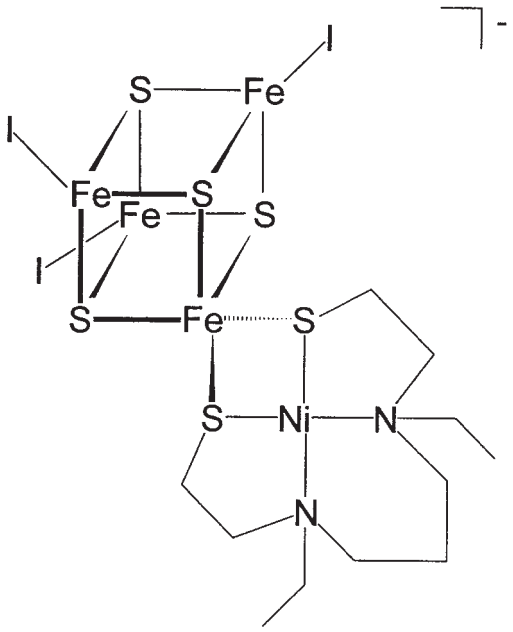


Fig. 7 The Ni-Fe_4S_4 assembly.

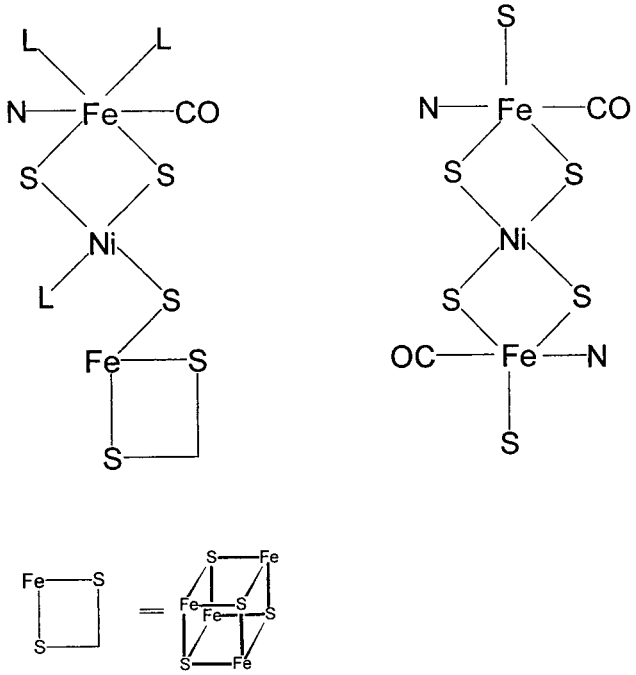


Fig. 8 A schematic representation of the proposed active centre of CODH from *R. rubrum* (left) and the core of complex **F** (right).

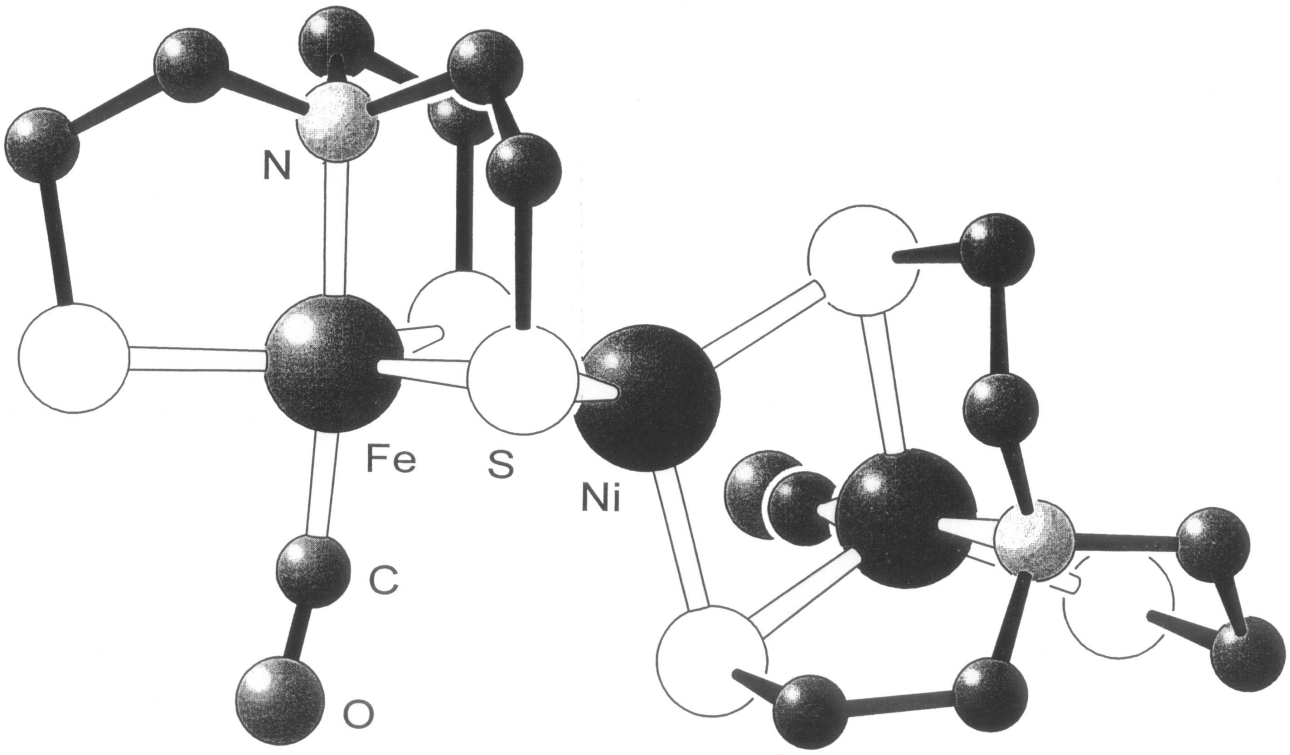


Fig. 9 A molecule of [Ni{Fe($N$$S_3$)(CO)-S,$S'$}$_2$], **F**, showing the principal atoms.

active site); and unusually, for nickel(II) in an all thiolate coordination environment, tetrahedral geometry about nickel.

Concluding Remarks

Directed inorganic synthesis is generating a range of complexes which have structural features similar to those of the active sites of various environmentally important enzymes. The spectroscopic characteristics of these complexes help in the understanding of the spectra obtained from the enzyme systems at various stages of turnover which, in turn, can extend our knowledge of the mechanism of action of the enzymes. The synthesis of complexes with an improved analogy to the active sites and a study of the reactivity of these complexes is expected to define new routes to the synthesis of novel, cheap, "green", catalysts.

Acknowledgement

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