Available online at www.sciencedirect.com
SCIENCE DIRECT®
Coordination Chemistry Reviews 249 (2005) 1641–1652
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

Iron-only hydrogenase: Synthetic, structural and reactivity studies of model compounds

Xiaoming Liu, Saad K. Ibrahim, Cédric Tard, Christopher J. Pickett *

Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

Received 7 February 2005; accepted 14 April 2005
Available online 1 June 2005

Contents

Abstract

The review reports progress in the chemistry of synthetic systems related to the active site of iron-only hydrogenase.

Keywords: Iron-only hydrogenase; Iron–sulfur clusters; Dihydrogen; Hydrides; Hydrogen economy; Thiolates

1. Introduction

Key questions which model studies of the active site of iron-only hydrogenase are beginning to address are: why are two metal centres necessary for the biocatalyses; why are the biologically unusual ligands CO and CN necessary structural and/or functional elements at the active sites; what is the mechanistic role of bridging/terminal CO interconversion during turn-over of Fe-only hydrogenase; what are the likely metal atom redox states involved in biocatalysis; and what are the minimum structural requirements necessary for synthetic analogues to display hydrogenase chemistry? Beyond providing chemical precedence for putative structures, intermediates and mechanisms in the biocatalyses, synthetic analogues of the active sites provide a means of validating interpretations of the spectroscopy of the enzyme systems and to 'ground' *in silico* DFT estimates of structural and spectroscopic properties of the active sites [1–3].

Earlier reviews [4–6] have discussed in depth some of these aspects of chemistry relating to Fe-only hydrogenase. We take these as a starting point but necessarily cover some of the same ground to place the subject in context and to provide a comprehensive background to the breaking research of the last 2 years in this rapidly moving area.

2. The structure of the catalytic site of Fe-only hydrogenase

X-ray crystallographic structures of Fe-only hydrogenases from *Desulfovibrio desulfuricans* and *Clostridium pasteuri-*

* Corresponding author.
E-mail address: chris.pickett@bbsrc.ac.uk (C.J. Pickett).

0010-8545/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2005.04.009

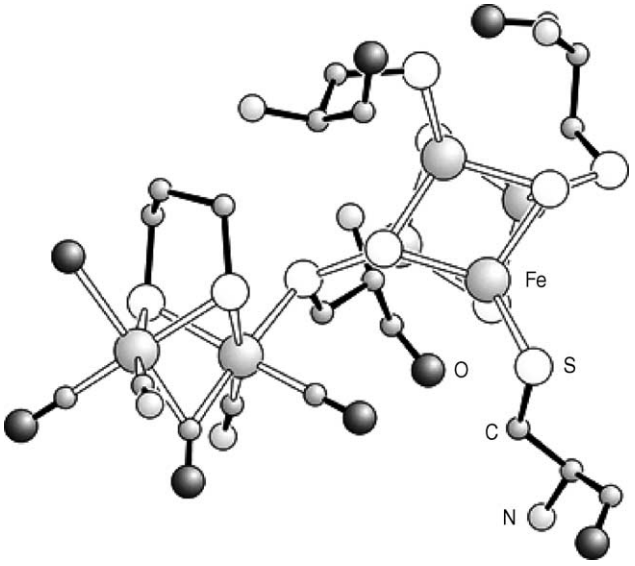


Fig. 1. Composite structure of the H-cluster constructed from the crystal structures of Fe-only hydrogenase isolated from *D. desulfuricans* (Code 1HFE) [7] and *C. pasteurianum* (Code 1FEH) [8] and FTIR data from *D. vulgaris*. The apical group on the subsite ligand may possibly be an NH or O but this remains crystallographically and analytically unresolved.

anum, together with spectroscopic data on Fe-only hydrogenase from *Desulfovibrio vulgaris*, show that the H-cluster, the active site at which protons are reduced to dihydrogen (or dihydrogen oxidised to protons) can be viewed as a conventional {4Fe4S}-cluster linked by a protein backbone bridging cysteinyl sulfur ligand to a {2Fe3S}-subsite (Fig. 1) [7–9].

At the subsite a terminal carbon monoxide, a bridging carbon monoxide and a cyanide ligand are bound at each iron atom which also share two bridging sulfur ligands of a 1,3-propanedithiolate or possibly related di(thiomethyl)oxo or di(thiomethyl)amine units. The first structural analyses of iron-only hydrogenases marginally favoured the latter ligand and led to the speculation that the amine group could function in proton delivery to the active site (Scheme 1) [3].

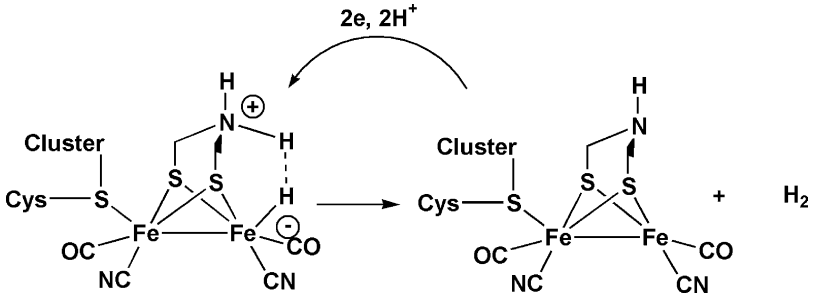
However, a recent higher resolution structure of Fe-only hydrogenase from *C. pasteurianum* has not allowed discrimination between NH, CH_2 or O at the '2-position' of the bridging dithiolate, although the earlier arguments favouring NH on the basis of hydrogen bonding interactions remain cogent.

The Fe-atom distal to the {4Fe4S}-cluster has a coordinated water molecule (or vacancy) in the resting paramagnetic oxidised state of the enzyme, $\{H_{ox}\}$. This site is occupied by carbon monoxide in the CO inhibited form of the enzyme $\{H_{ox}(CO)\}$ and this is where hydride/dihydrogen are likely to be bound during turnover [3,10]. For neither the iron-only or the nickel only hydrogenases is their direct spectroscopic evidence for metal hydrides, although photolysis and H/D isotope studies on the Ni–C state of the latter provide strong indirect evidence [11,12].

It has been perhaps tacitly assumed that the iron–sulfur cluster linked by the bridging cysteinyl unit to the diiron subsite 'merely' functions as a benign electron-transfer unit. In detail this may be a simplification. Unless the two components are essentially 'insulated', changing the redox state on the cluster would be expected to induce changes in the electron density at the subsite and this would impinge on reactivity. Some recent work has suggested that the μ-S linked cluster is non-innocent in its interaction with the juxtaposed diiron unit, as is discussed below.

3. Synthetic models of the diiron subsite

The crystallographic characterisations of iron-only hydrogenase revealed the close resemblance of the subsite of the H-cluster to known $[Fe_2(\mu\text{-}SR)_2(CO)_6]$ (R = organic group) complexes. This type of {2Fe2S}-assembly, first reported [13] three quarters of a century ago and developed further half a century later [14] paved the way for the synthesis of subsite models. Three groups independently reported the dianion $[Fe_2(SCH_2CH_2CH_2S)(CO)_4(CN)_2]^{2-}$ **1** (Fig. 2, and Scheme 2) [15–17] soon after publication of the enzyme crystallographic data. Impressive work by Rauchfuss and co-workers [18] showed that the related $[Fe_2(SCH_2NRCH_2S)(CO)_4(CN)_2]^{2-}$ (R = H, Me) dianions **2** are also accessible by a different route as also shown in Fig. 2 and Scheme 2. Subsequently, Song and co-workers showed the oxo analogue $[Fe_2(SCH_2OCH_2S)(CO)_4(CN)_2]^{2-}$ **3** can be made in a similar way [19] (Scheme 2). The 'butterfly' arrangement of the dithiolate ligands in both **1**, **2** and **3** are closely similar to that in the enzymic subsite (Fig. 1) and the $\{Fe(CO)_2(CN)\}$ motifs in the complexes reasonably model



Scheme 1. Proposed role of putative bridging NH in enzymic hydrogen evolution.

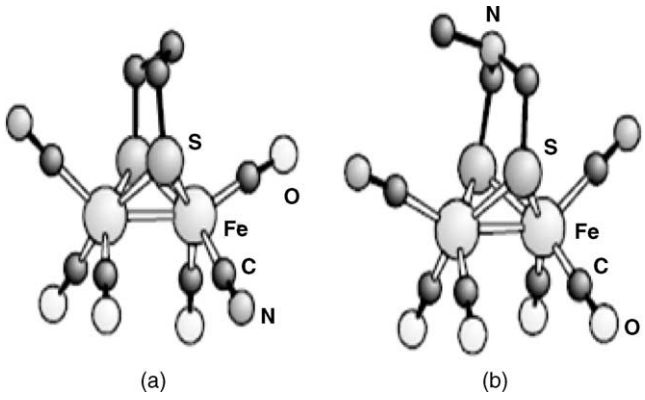


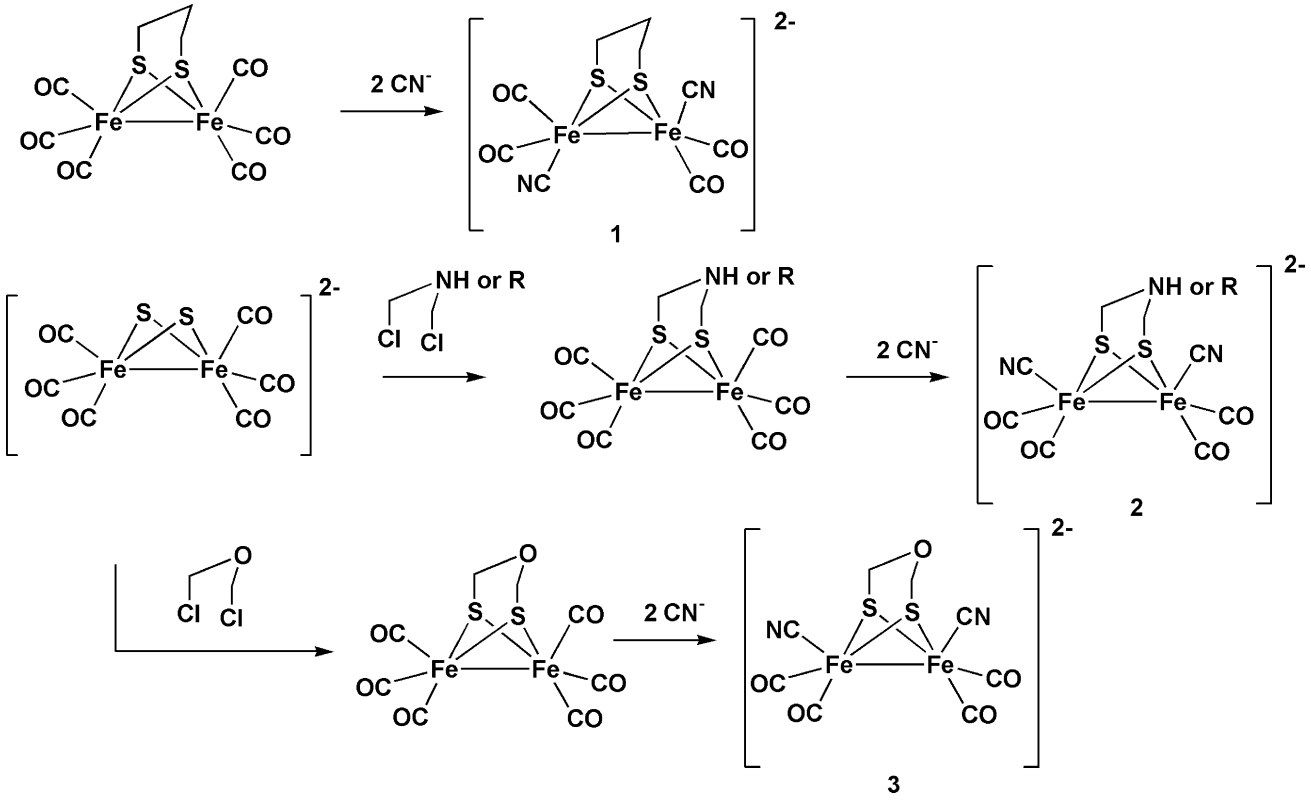
Fig. 2. Analogues of the subsite of the H-cluster possessing a 2Fe2S-framework and CO/CN ligation. The analogues have structural features of CO inhibited form of the enzyme. Notice the different positional geometry of CN and CO ligands in complex (a) compared with (b). Reprinted with permission of the Royal Society of Chemistry (ref. [5]).

the distal iron of the subsite in the CO inhibited form of the enzyme $\{H_{ox}(CO)\}$.

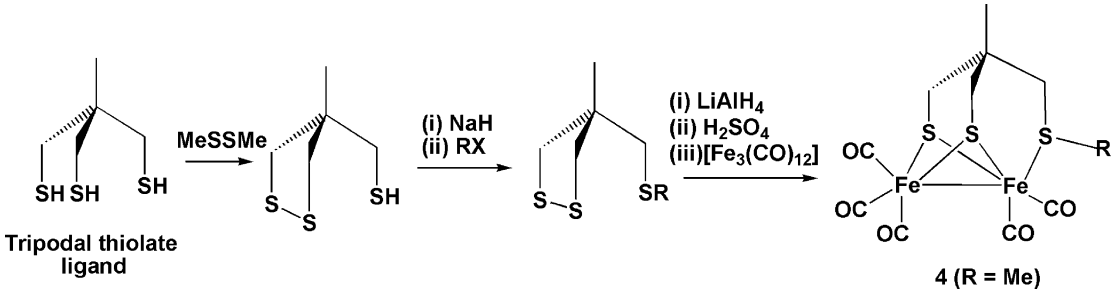
Darensbourg and co-workers [20] have shown that the monocyanide $[Fe_2(SCH_2CH_2CH_2S)(CO)_5(CN)]^-$ can be indirectly synthesised by nucleophilic attack of $[(Me_3Si)_2NLi]$ on $[Fe_2(SCH_2CH_2CH_2S)(CO)_6]$, that it reacts with CN^- to give **1**, and is also detected by FTIR as an intermediate in the overall substitution pathway. The monocyanide is therefore a plausible intermediate in stepwise substitution of the parent hexacarbonyl which gives the di-cyanide **3**. However, it has also been suggested that there is an alternative di-cyanation pathway which proceeds via fast attack on an (undetected) bridging CO monocyanide intermediate, a precursor to the monocyanide [21]. The evidence for this is that the rate of formation of **1** from the parent hexacarbonyl and CN^- is *faster* than it is directly from the monocyanide. The mechanism of cyanation of {2Fe3S}-carbonyls has been studied in detail both experimentally and also *in silico* as is discussed below [22].

Complexes **1**, **2** and **3** have essentially undifferentiated ligation at the two – iron sites and can be viewed as possessing a {2Fe2S} – rather than the {2Fe3S}-unit as is observed within the protein. Pickett and co-workers [23] have described the synthesis of propanedithiolate ligands functionalised with an appended thioether group and showed that these allow the assembly of {2Fe3S}-carbonyls such as $[Fe_2\{MeSCH_2C(Me)(CH_2S)_2\}(CO)_5]$ **4** (Scheme 3). X-ray crystallography of **4** revealed the close similarity of the {2Fe3S}-unit in the synthetic model and that in enzyme (Fig. 3) [23,24].

The pentacarbonyl **4** (R = Me) is readily converted to a stable monocyanide derivative **6** in which cyanation takes place regioselectively at the Fe atom distal to the thioether ligand (Fig. 3, Scheme 4). The reaction of **6** with further cyanide results in the formation of a moderately stable intermediate $[Fe_2\{MeSCH_2C(Me)(CH_2S)_2\}\mu(CO)(CO)_3(CN)_2]^{2-}$ **7** possessing a bridging CO, a cyanide ligand at each Fe atom,



Scheme 2. Synthesis of {2Fe2S}-subsite analogues.



Scheme 3. Synthesis of {2Fe3S}-complexes. The synthetic pathway offers considerable scope for varying the functionality R.

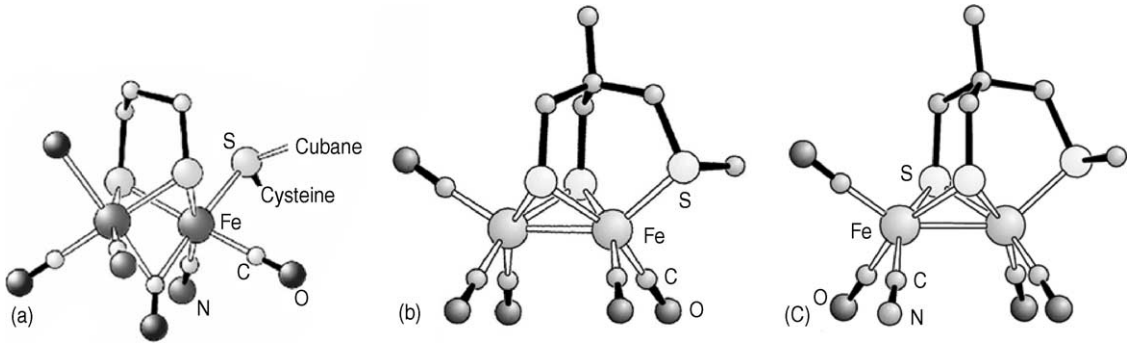
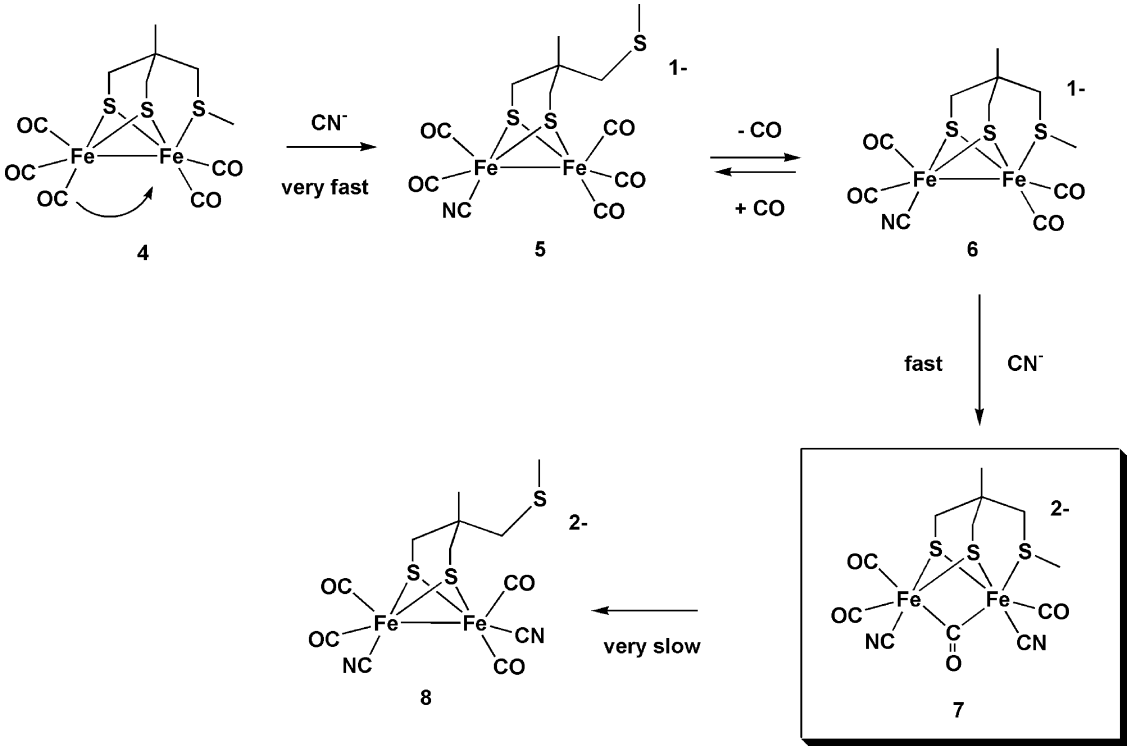


Fig. 3. (a) Proposed structure of the H-cluster of Fe-only hydrogenases; this is a composite model combining features reported by Nicolet (PDB code 1HFE) [7] and Peters (code 1FEH) [8] and (b) complex **4**, (c) monocyanide derivative complex **6**. Reprinted with permission of the Royal Society of Chemistry (ref. [5]).



Scheme 4. The cyanide substitution pathway of {2Fe3S}-subsite as deduced from stopped-flow FTIR kinetic analysis and labelling studies.

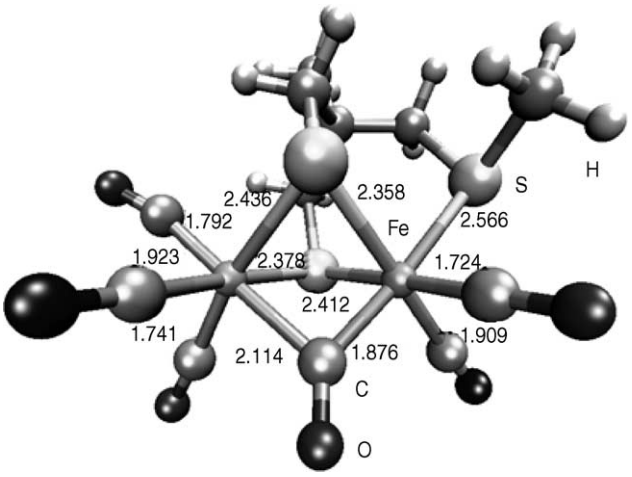


Fig. 4. DFT structure of bridging carbonyl intermediate **9** which possesses the {2Fe3S}-framework of the subsite of the H-cluster (CO inhibited form).

and differential ligation at the Fe atoms. These are key structural elements of the CO inhibited subsite of the H-cluster. The intermediate **7** slowly rearranges to **8**, a close {2Fe2S}-analogue of **1** (Scheme 4). The detailed mechanism of cyanation of the carbonyl **4** has been studied by stopped-flow FTIR and UV–vis spectroscopy [22]. This has: shown 'on-off' thioether ligation plays a critical role in the primary substitution step; unequivocally defined the monocyanide **5** as an intermediate; and shown the bridging carbonyl species **7** is on the pathway to **8** (Scheme 4). The intimate mechanism of this substitution pathway has now been dissected *in silico* by DFT studies [25]. This has provided much insight into the structure of energetically plausible transition states and provided calculated infrared CO and CN frequencies which correlate very well with detected intermediates and products, as shown for $Fe_2\{MeSCH_2C(Me)(CH_2S)_2\}\mu(CO)(CO)_3(CN)_2]^{2-}$ (Fig. 4) [26].

4. Biosynthesis of the subsite, chemical precedents and pre-biotic speculation

How the subsite of iron-only hydrogenase is intracellularly synthesised is far from established. The origin of the cyanide ligands at Fe in NiFe-hydrogenases and possibly in the iron-only hydrogenases is carbamoyl phosphate. The NiFe-hydrogenases have an Ni–Fe site in which the iron has one CO and two CN groups as ligands. In elegant studies by Bock and co-workers [27] it has been shown that the synthesis of the CN ligands requires the activity of two hydrogenase maturation proteins: HypF and HypE. HypF is a carbamoyltransferase that transfers the carbamoyl moiety of carbamoyladenylate to the COOH-terminal cysteine of HypE and thus forms an enzyme-thiocarbamate. HypE dehydrates the *S*-carbamoyl moiety in an adenosine triphosphate-dependent process to yield the enzyme thiocyanate.

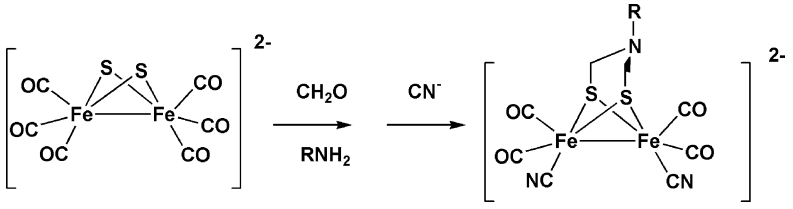
Chemical model reactions corroborate the feasibility of this unprecedented biosynthetic route and show that thiocyanates can donate CN to iron. This finding underscores a striking parallel between biochemistry and organometallic chemistry in the formation of an iron–cyano complex. Earlier studies [28] suggested that CO ligands at Fe also originate from carbamoyl phosphate but so far there is no biochemical evidence to support this.

All organisms with iron-only hydrogenase and sequenced genomes contain homologues of HydE, HydF, and HydG and in several these are found in putative operons with iron-only hydrogenase structural genes. Both HydE and HydG belong to the emerging radical *S*-adenosylmethionine (commonly designated the "Radical SAM") super family of proteins and it has recently been confirmed that HydEF and HydG function in the assembly of iron-only hydrogenase as accessory genes required for the maturation of the active enzyme [29].

Cody et al [30] have shown that, among other iron carbonylated species, organometallic $[Fe_2(\mu\text{-}SR)_2(CO)_6]$ derivatives can be generated from a simple chemical soup containing iron sulfide, alkanethiol and formic acid (a source of CO) possibly extant in prebiotic times and this provides the basis of speculation on a pre-biotic role for diiron carbonyl thiolates. It is not yet known whether formate is the source of CO in the biological systems but dehydration of HCOOH, H-radical abstraction from (or 2e oxidation of HCHO) could provide CO. Somewhat related to this is novel chemistry described by Li and Rauchfuss [31] which utilises a 'one-pot' formaldehyde and amine chemistry to assemble azapropane dithiolate complexes — the carbon units of the bridge originate from HCHO (Scheme 5).

5. Oxidation-states of the diiron subsite

The oxidation states the two Fe centres in the subsite of the H-cluster have been the subject of some considerable discussion [9,32,3]. In the EPR active $\{H_{ox}\}$-state, the proximal



Scheme 5. 'One-pot' syntheses of azathiolate complexes.

iron-atom is almost certainly diamagnetic Fe(II) because only weak magnetic coupling of the paramagnetic {4Fe4S} cluster to the subsite is observed [32,34]. Thus the spin-density in the subsite resides on the distal Fe-atom. The question is whether this metal atom has a formal oxidation state of Fe(III) or is in a biologically unprecedented Fe(I) state. A Mössbauer study assigned the oxidation state of the distal iron as (conventional) Fe(III) state but the authors added a caveat that Fe(I) could not be excluded, particularly in the light of chemical syntheses of Fe(I)–Fe(I) subsite analogues [32,35]. However, FTIR ^{13}CO labelling studies of the enzyme by DeLacey et al. [9] strongly support an Fe(I) oxidation state for the distal iron centre in $\{H_{ox}(CO)\}$ because the uncoupled ν(CO) stretch at this centre is *lower* in energy than that for the CO bound at the proximal Fe(II) site. Strong support for an $\{Fe(I)_{distal}–Fe(II)\}$ arrangement comes from studies of synthetic {2Fe3S}-systems [22,35] and from DFT calculations [36] and this is now the generally accepted oxidation-state arrangement of the subsite.

Although the intermediate **7** possesses structural elements of the subsite of $\{H_{ox}(CO)\}$, electronically it differs in being a diamagnetic Fe(I)–Fe(I) rather than a paramagnetic Fe(I)–Fe(II) or Fe(III)–Fe(II) system. Generating paramagnetic species by oxidation of precursors such as **1**, **2** or **8** has been problematic, no stable mixed valence species have been isolated. Best, Pickett and co-workers [35] have shown that a transient Fe(I)–Fe(II) species can be spectroscopically characterised using FTIR spectroelectrochemical and stopped-flow methods. Thus one-electron oxidation of **8** generates a short-lived paramagnetic species with a bridging CO group. This species has ν(CO) bands at wavenumbers close to those of $\{H_{ox}(CO)\}$ (Scheme 6). This supports the work of DeLacey and co-workers who analysed ^{13}CO/^{12}CO FTIR spectra of iron-only hydrogenase [9] and concluded that the distal Fe atom of the subsite was in the lowest oxidation state and because the spin density is also located on the distal Fe centre, as deduced from magnetic studies by Munck and co-workers [32], then this is Fe(I), i.e. $\{H_{ox}\}$ and $\{H_{ox}(CO)\}$ states of the enzyme have a mixed valence Fe(I)–Fe(II) unit [32,33].

In silico DFT calculations on model assemblies by Cao and Hall [37], and subsequently by Liu and Hu [38], also conclude that $\{H_{ox}\}$ and $\{H_{ox}(CO)\}$ most likely consist of an Fe(I)–Fe(II) pair (see Chapter by De Gioia et al. [26]). Taken together the enzymic, model complex and DFT calculations all point to an unexpected role for Fe(I)–Fe(I)/Fe(I)–Fe(II) systems in biology. It is the π-acid ligands, CO and CN, which undoubtedly allow access to these low oxidation/spin-state levels.

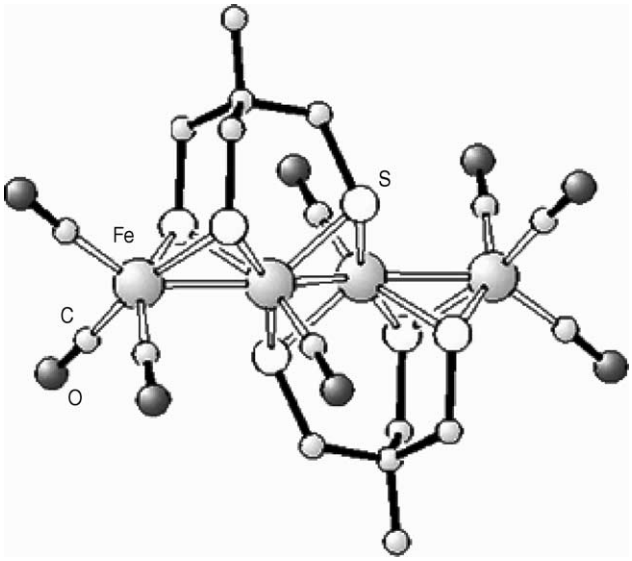
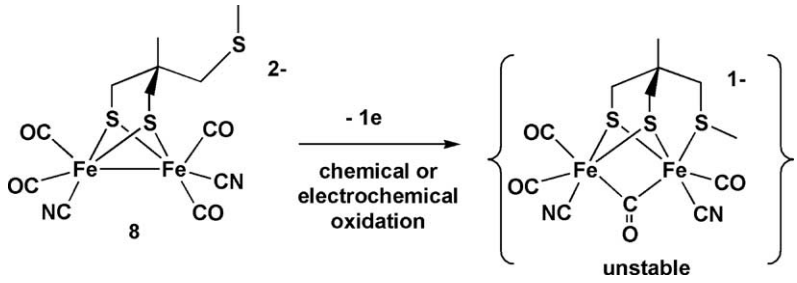


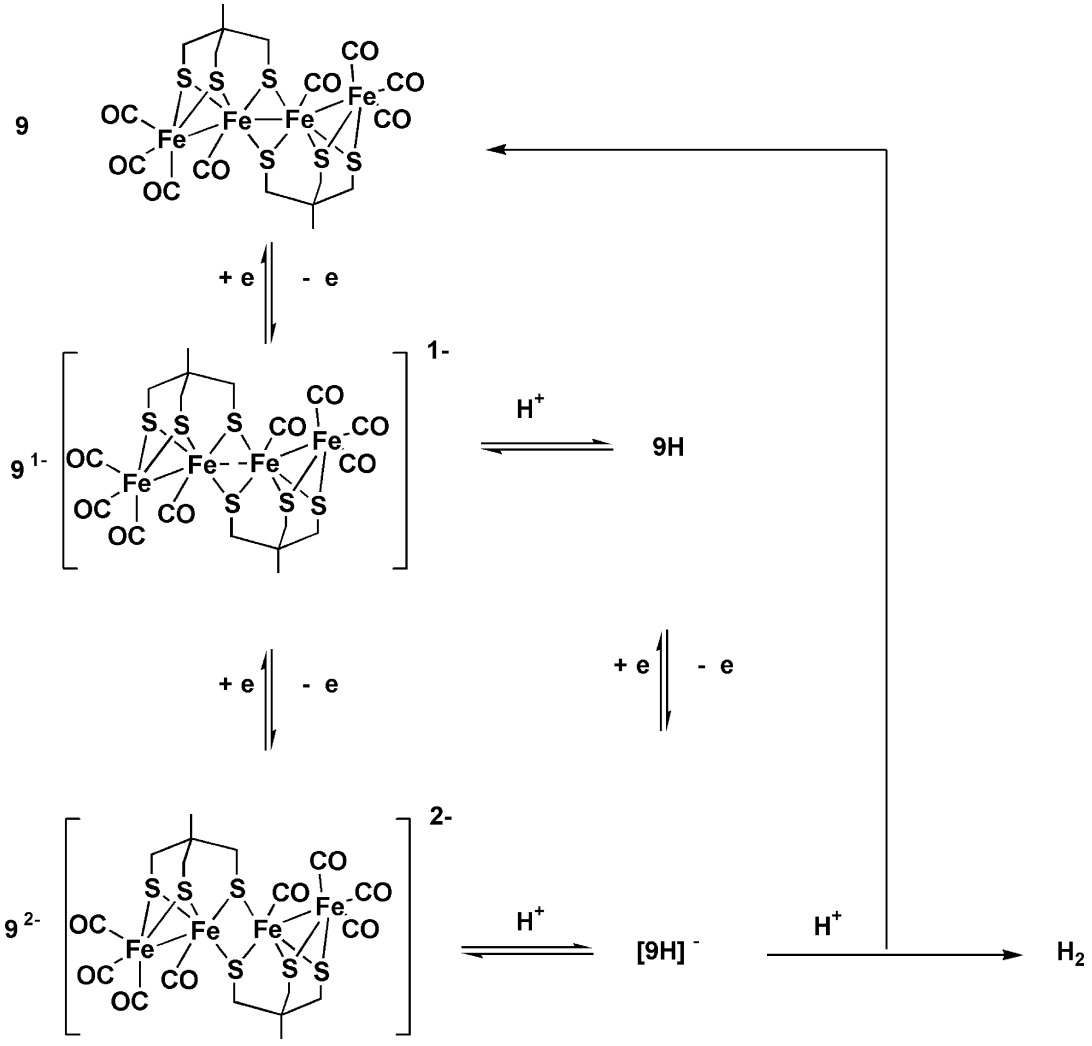
Fig. 5. A view of the structure of **9**. The two exo-Fe atoms are formally Fe(I) and the two internal Fe atoms are formally Fe(II).

Although stable higher oxidation state diiron units with CO/CN co-ligands remain elusive, Rauchfuss and co-workers [39] have shown that electron-rich Fe(II)–Fe(II) polyisocyano-derivatives can be synthesised. For example, $[Fe_2\{SCH_2CH_2CH_2S\}(CNMe)_7][PF_6]_2$ in which one isocyanide ligand bridges the metal atoms has been crystallographic characterized. The 34e(−) dinuclear core has some resemblance to the oxidized (H_2-binding) form of the active sites of the Fe-only hydrogenases, key features being the face-sharing bioctahedral geometry, the (μ-CX) ligand, and an Fe–Fe separation of 2.61 Å.

A stable tetra-nuclear system **9** (Fig. 5) which possesses two 'fused' {2Fe3S}-subsite units has recently been reported has the two 'sets' of iron atoms formally in the Fe(I)–Fe(II) oxidation states. Notably this structure possesses only CO co-ligands and has been shown to electrocatalyse proton reduction at moderate overpotentials (Scheme 7 and Fig. 5) [40].



Scheme 6. Generation of Fe(I)–Fe(II) mixed valence species.



Scheme 7. Electrocatalysis of proton reduction by **9**.

6. Hydrides and H/D exchange reactions

Implicit in hydrogen uptake/evolution by the hydrogenase is the formation of metal–hydride and dihydrogen bonds. However, there is no direct spectroscopic data establishing the presence of metal–hydride (M–H) bonds nor direct evidence for dihydrogen intermediates in biological catalysis by the hydrogenases, nor indeed other enzymic systems which interconvert H^+/H_2, such as the nitrogenases [41]. This is perhaps not too surprising since strong M–H bonds would be an anthema to fast catalysis as would stable enzymic metal–(H_2) intermediates. The indirect biological evidence for hydride and dihydrogen intermediates rests largely with the formation of HD during enzymic turnover under an atmosphere of D_2 and/or with the formation of HDO under this gas [34]. However, such observations do not unequivocally eliminate H/D exchange chemistry taking place at S or even at CN sites via formation of aminocarbenes [42], however unlikely this may seem.

Protonation of the subsite analogue $[Fe_2(SCH_2CH_2CH_2S)(CO)_4(CN)_2]^{2-}$ **1** gives some dihydrogen together with uncharacterisable metal products [16]. Seminal work by Poilblanc and co-workers some 25 years ago showed that certain diiron bis-thiolate and bis-phosphido carbonyl species protonate on the metal–metal bond [43]. The X-ray structures of $[Fe_2(SCH_2CH_2CH_2S)(\mu\text{-}H)(CO)_4(PMe_3)_2]^+$ **10** and several new bridging thiolate phosphino complexes has been reported by Darensbourg and co-workers [44,45]. Structural comparison found little difference in the {2Fe2S} butterfly cores, with Fe–Fe distances of 2.555(2) and 2.578(1) Å for the neutral parent species $[Fe_2(SCH_2CH_2CH_2S)(CO)_4(PMe_3)_2]$ and the protonated bridging hydride species **10**, respectively. Both are similar to the average Fe–Fe distance found in structures of three Fe-only hydrogenase active site {2Fe2S} clusters: 2.6 Å.

H/D exchange reactions have been used to assay hydrogenase activity [12,46,47]. Darensbourg and co-workers have been at the forefront of elucidating H/D exchange

reactions of model compounds with μ-hydrides. In a reaction that is promoted by light but inhibited by CO, $[Fe_2(SCH_2CH_2CH_2S)(\mu\text{-}H)(CO)_4(PMe_3)_2]^+$ **10** shows H/D exchange activity with D_2 producing in CH_2Cl_2 and in acetone $[Fe_2(SCH_2CH_2CH_2S)(\mu\text{-}D)(CO)_4(PMe_3)_2]^+$, but not in CH_3CN. In the presence of light, H/D scrambling between D_2O and H_2 is also promoted in the presence of **10**. The requirement of an open site generated by photolytic CO loss has led to the suggestion that the key step in the reactions involves D_2 or H_2 binding to an Fe centre followed by deprotonation by the internal base, or by external water. As indicated by similar catalytic efficiencies of members of the series, the nature of the bridging thiolates has little influence on the reactions. Comparison to the iron-only enzyme active site redox levels has led to further speculation that at least one Fe(II) site must be available for H_2 uptake while a reduced or an electron-rich metal–metal bonded redox level is required for proton uptake.

Whereas the dicyanide **1** gives ill-defined products on protonation[13], Rauchfuss and co-workers have shown that the monocyanide $[Fe_2(SCH_2CH_2CH_2S)(CO)_4(PMe_3)(CN)]^-$ **11** protonates at the metal–metal bond giving $[Fe_2(SCH_2CH_2CH_2S)(\mu\text{-}H)(CO)_4(PMe_3)(CN)]$ **12** and the structure of this molecule has been determined crystallographically (Fig. 6) [48].

In the presence of a stronger acid further protonation of **12** at the cyanide ligand is believed to take place. On the basis of these observations it has been argued that formally replacing one cyanide in **1** by the weaker net electron-donor ligand PMe_3 stabilises the diiron unit with respect to further oxidation by proton, allowing isolation of the (μ-H) species. Similarly it has recently been shown that the bis-isocyanide complex $[Fe_2(SCH_2CH_2CH_2S)(CO)_4(^tBuNC)_2]$ which exists in multiple isomeric forms in solution also protonates at the metal–metal bond to give isomeric (μ-H) species. This protonated system does not react with D_2 at a pressure near 6.5 atm in the dark but when illuminated with visible light HD is formed [49].

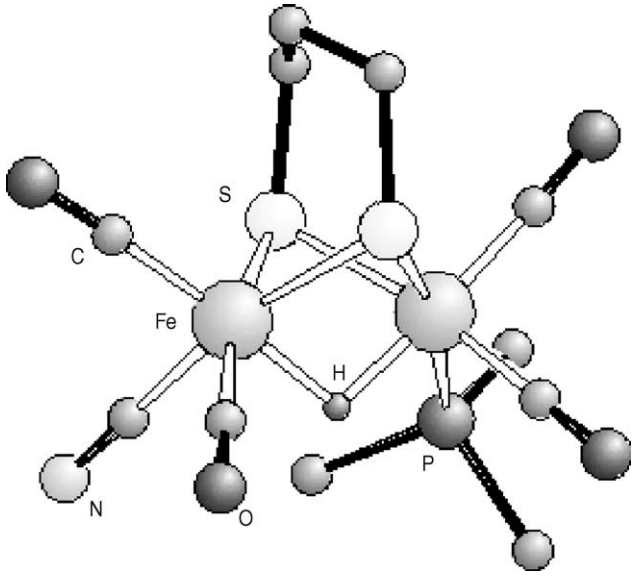
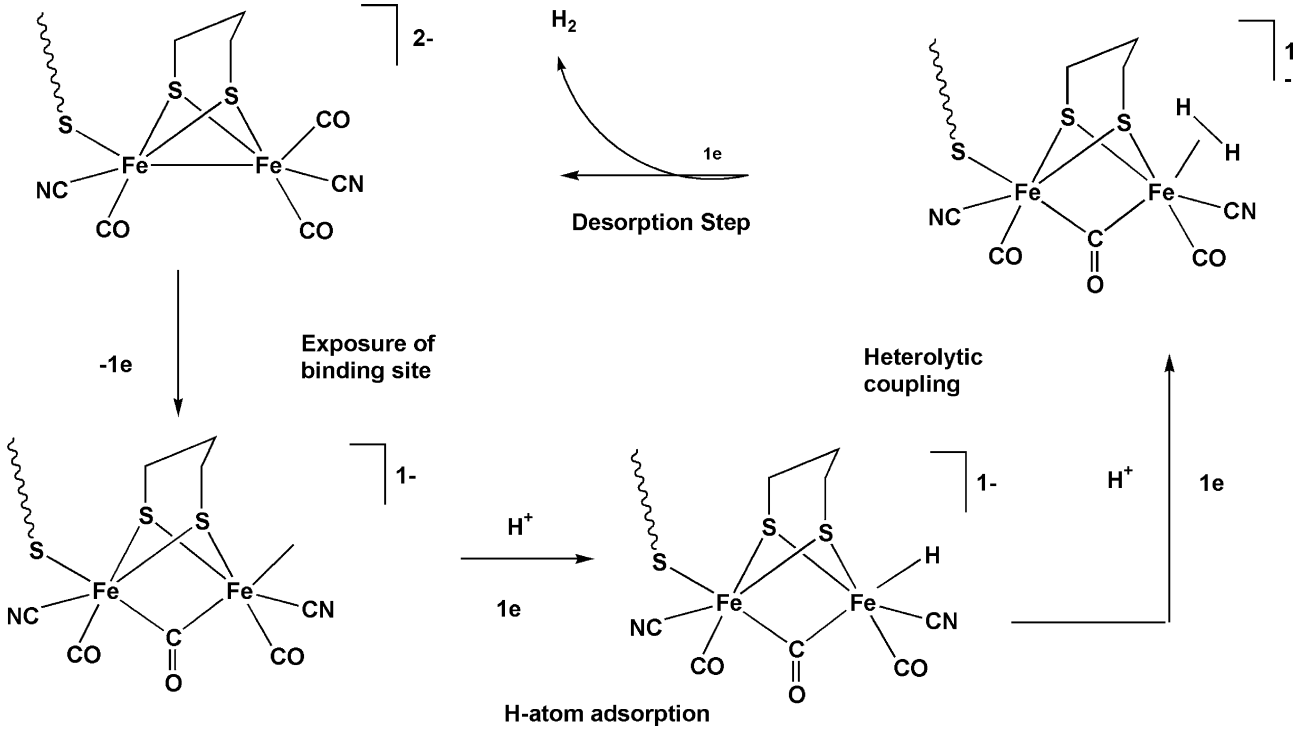
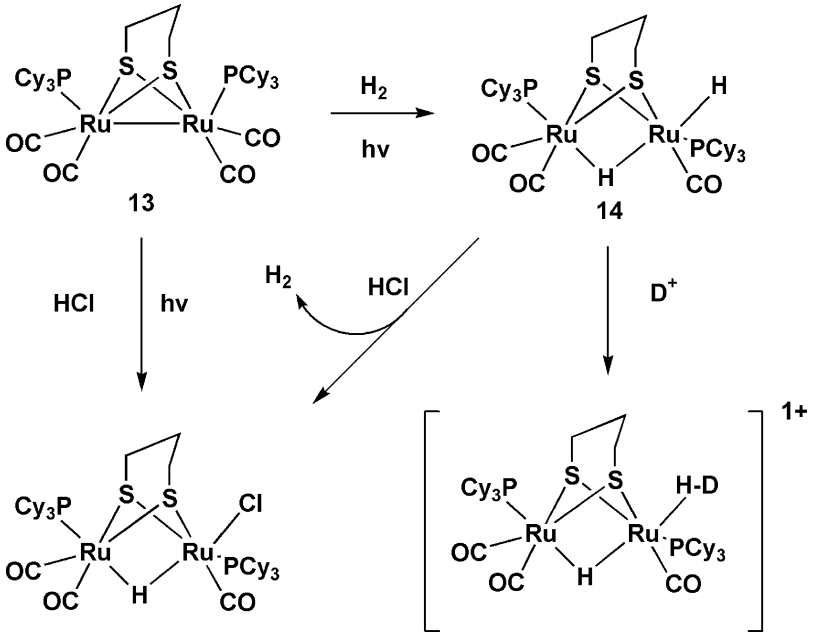


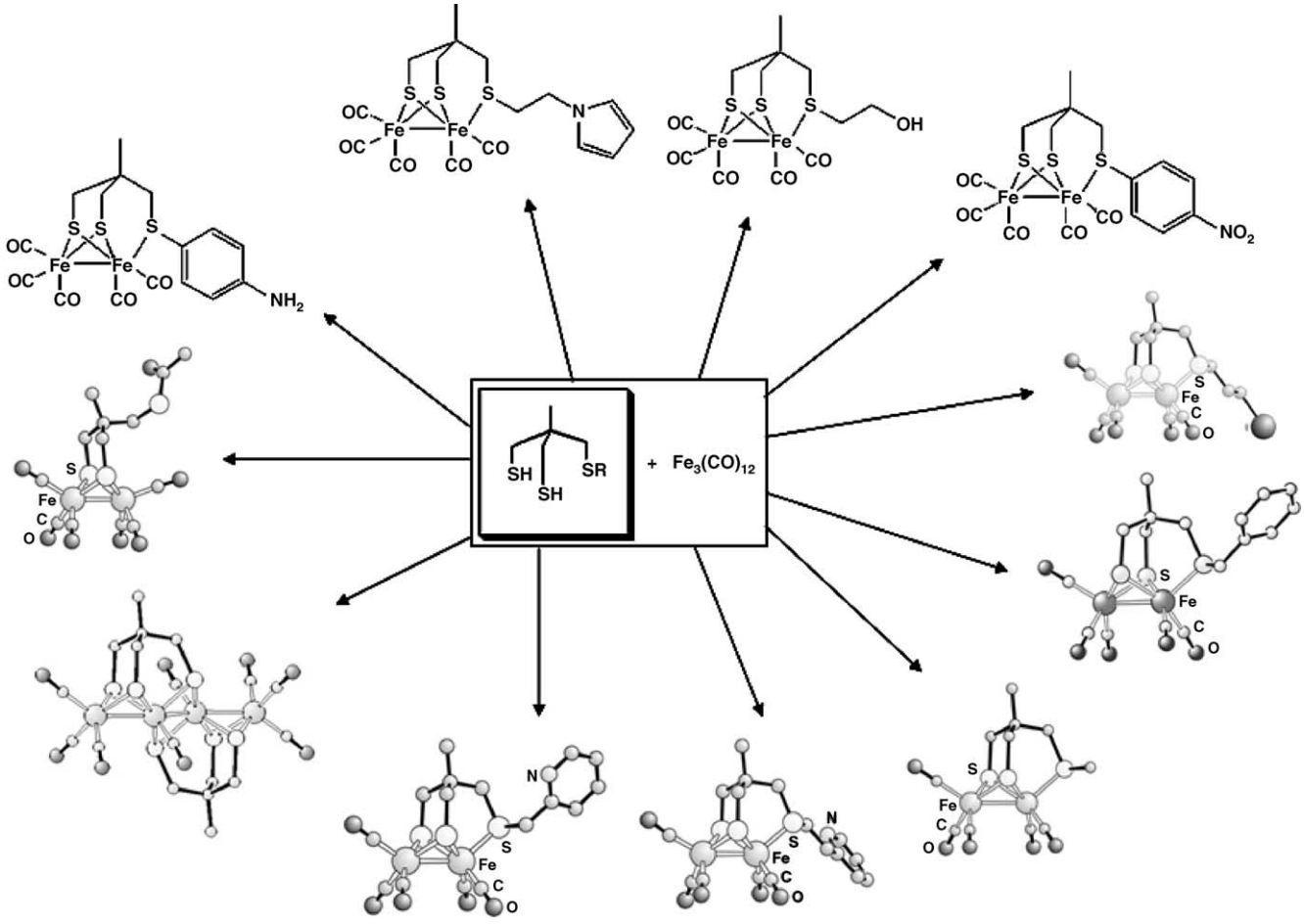
Fig. 6. X-ray structure of **12** showing the hydride ligand bridging the two iron atoms.



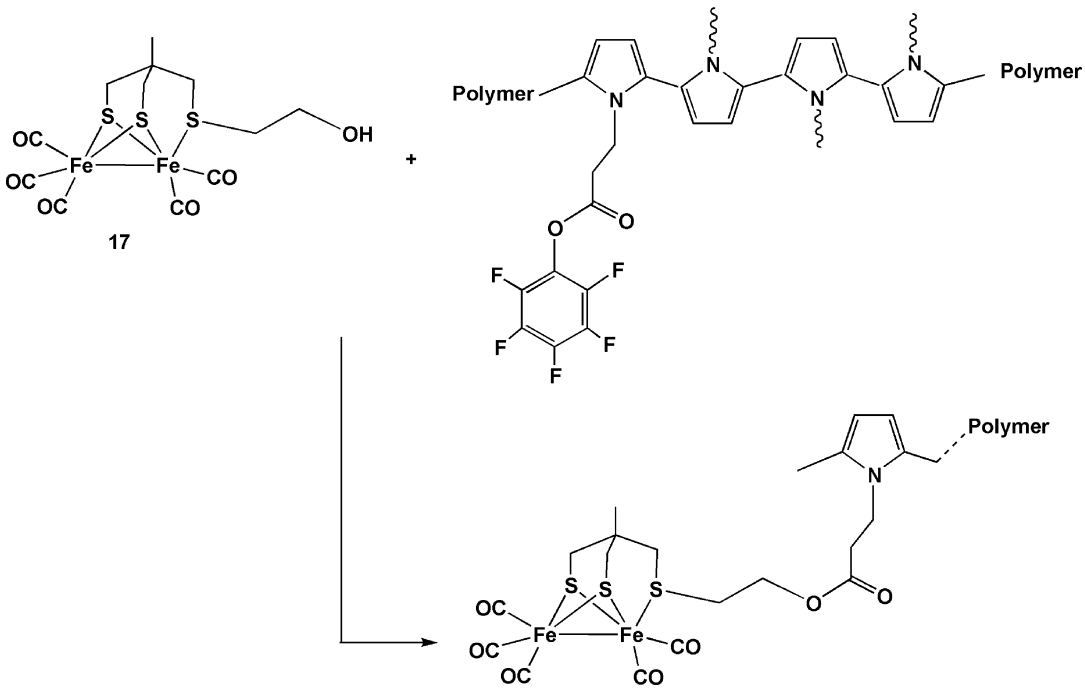
Scheme 8. Possible role of bridging CO in enzyme catalysis.



Scheme 9. Ruthenium dihydrogen/hydride chemistry.



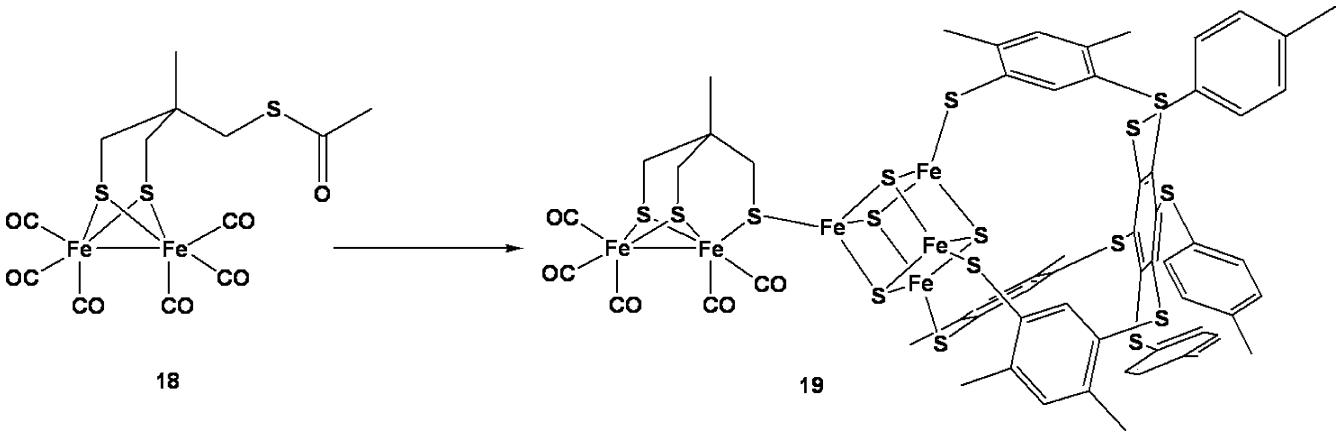
Scheme 10. Functionalisation of {2Fe3S} units.



Scheme 11. Anchoring {2Fe3S}-subsites within an electropolymer.

Whether or not (μ-H) intermediates are involved in the enzymic catalysis is not known. The vacancy or water occupied site on the distal iron atom in the resting state of the enzyme, and the inhibition of proton reduction when this site is occupied by CO, has led to postulates that a terminally bound hydride at this site is involved during turnover. It might well be that the role of *bridging CO* in the enzymic system is to prevent protonation at a Fe–Fe bond. It is becoming clear from FTIR studies on the enzyme system that the bridging CO of $\{H_{ox}\}$ becomes terminally bound on reduction [50]. The mechanistic implications of this may be related to the exposure of a proton binding site at the distal iron when CO adopts the bridging mode, with subsequent elimination of dihydrogen driven by rearrangement to a terminal CO bonding mode with concomitant metal–metal bond formation (Scheme 8). This scheme invokes formation of terminal hydride/dihydrogen intermediates.

Synthetically, terminal hydride/dihydrogen diiron dithiolate model complexes have proved elusive but Rauchfuss and co-workers have recently have made two significant advances in the generally area. First, they have shown that a close analogue of $[Fe_2(SCH_2CH_2CH_2S)(CO)_4(PMe_3)_2]$, the ruthenium complex $[Ru_2(SCH_2CH_2CH_2S)(CO)_4(PCy_3)_2]$ (Cy = cyclohexyl) **13** can be photolysed under dihydrogen to eliminate CO and cleave dihydrogen to give a complex with *both* a terminal and a bridging hydride ligand, $[Ru_2(SCH_2CH_2CH_2S)(\mu\text{-}H)H(CO)_3(PCy_3)_2]$ **14**. Treatment of **13** with a non-coordinating acid under D_2 leads to formation of HD but the (μ-H) ligand is inert to exchange. Treatment with HCl leads to release of dihydrogen by



Scheme 12. Synthesis of the iron–sulfur framework of the H-cluster.

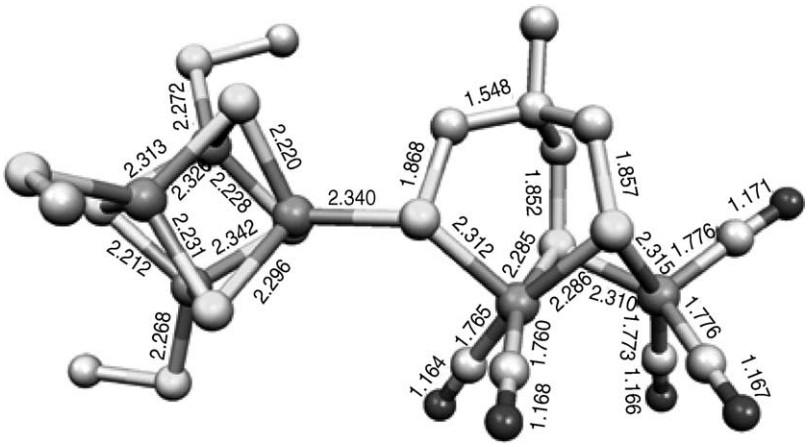


Fig. 7. DFT structural model of an MeS analogue of **19**.

protonation of the more hydridic terminal hydride with formation of $[Ru_2(SCH_2CH_2CH_2S)(\mu\text{-}H)Cl(CO)_3(PCy_3)_2]$. These reactions are summarised in Scheme 9. Secondly, the first Fe hydride–carbonyl–cyanide has been synthesised albeit in a mononuclear system. *Trans*-$[Fe(CN)_2(CO)_3]^{2-}$ **15** forms in high yield via photosubstitution of $Fe(CO)_5$ with 2 equiv. of $[Et_4N]CN$. Protonation of **15** generates the hydrido-species $[HFe(CN)_2(CO)_3]^-$ which on further reaction with acid liberates dihydrogen.

Terminal hydrido-intermediates formed during reduction of $[Fe_2(PPh_2)_2(CO)_6]$ a phosphido analogue of **1** in the presence of protons have been characterised by spectro-electrochemical, continuous-flow, and interrupted-flow techniques. A mechanism for electrocatalytic proton reduction has been proposed with digital simulation of the voltammetry in close agreement with measurements conducted in THF over a range of acid concentrations. It is proposed that the electrocatalysis involves initial formation of the dianion, $[Fe_2(PPh_2)_2(CO)_6]^{2-}$ which is doubly protonated prior to further reduction and dihydrogen elimination [51].

7. Functionalised diiron dithiolate units and approaches to the synthesis of the H-cluster

An important research avenue which is being widely explored is the functionalisation of subsite analogues for purposes such as attaching neighbouring redox centres, photocentres, or for anchoring groups to electrode surfaces or proteins and for water solubility [52]. Such approaches may afford new systems for redox or photoredox catalysis [53] of hydrogen/evolution uptake and further insight into the biological chemistry.

One of the first types of functionality to be attached to a diiron subsite type unit were C_{60} and C_{70} fullerenes by irradiation in the presence of $[Fe_2(S)_2(CO)_6]$ and related phosphine substituted species. As expected the fullerene units display multiple electron-transfer chemistry and it will be interesting to see developments in this area with respect to electrocatalysts [54].

Various groups R have been attached to the N azapropane dithiolate complexes of the type $Fe_2[(SCH_2)_2NR](CO)_6$ by the route shown in Scheme 2 including R = $CH_2CH{=}CH_2$ and CH_2CH_2SMe. Me_3NO-induced decarbonylation of these afforded pentacarbonyl derivatives wherein the pendant functionality is coordinated to one Fe atom [55]. Free carboxylate and amine groups have also been attached as potential conjugates with protein groups by a similar approach [56].

The Bosnich ligand $CH_3C(CH_2SH)_3$ [57] has offered much scope for elaboration of {2Fe3S}-units as outlined in Scheme 10. The pyrrole derivative **16** does not electropolymerise because it is destructively oxidised but the alcohol **17** can be reacted with an active ester electropolymer [58,59] to allow incorporation of {2Fe3S}-subsites into an electrode bound polymer film (Scheme 11).

Finally, the actived *thioester* complex **18** has been used to build the first synthetic analogue possessing the iron–sulfur framework of the complete H-cluster of iron-only hydrogenase **19** as shown in Scheme 12. The DFT structure of a methylthiolate analogue of **19** is shown in Fig. 7 [59]. Studies of the electron-transfer chemistry of assemblies such as **19** are beginning to provide insight into the interplay of subsite and cluster units and are perhaps indicating that the latter may not behave 'merely' as a spectator electron-transfer unit.

8. Conclusions

Chemistry related to the iron-only hydrogenase is developing very rapidly and providing some informative insights into how the biological system might function. Although still very much at a blue skies stage there is some prospect for the design of assemblies, materials and devices with technological application for hydrogen production/uptake in fuel cells provided systems can be designed with low overpotentials for hydrogen/proton interconversion. So far, the synthetic diiron

subsite analogues have only been shown to display electrocatalysis of proton reduction and this at very negative potentials [60]. However, with efforts to synthesise materials which more closely model the natural site, in particular anionic dicyanide Fe(I)–Fe(II) systems with a coordinatively unsaturated or weakly ligated distal iron atom, potentially useful electrocatalysts might be achieved.

References

[1] F.A. Armstrong, Curr. Opin. Chem. Biol. 8 (2004) 133.
[2] Y. Nicolet, C. Cavazza, J.C. Fontecilla-Camps, J. Inorg. Biochem. 91 (2002) 1.
[3] Y. Nicolet, B.J. Lemon, J.C. Fontecilla-Camps, J.W. Peters, Trends Biochem. Sci. 25 (2000) 138.
[4] M.Y. Darensbourg, E.J. Lyon, J.J. Smee, Coord. Chem. Rev. 206 (2000) 533.
[5] D.J. Evans, C.J. Pickett, Chem. Soc. Rev. 32 (2003) 268.
[6] T.B. Rauchfuss, Inorg. Chem. 43 (2004) 14.
[7] Y. Nicolet, C. Piras, P. Legrand, C.E. Hatchikian, J.C. Fontecilla-Camps, Struct. Fold. Des. 7 (1999) 13.
[8] J.W. Peters, W.N. Lanzilotta, B.J. Lemon, L.C. Seefeldt, Science 282 (1998) 1853.
[9] A.L. De Lacey, C. Stadler, C. Cavazza, E.C. Hatchikian, V.M. Fernandez, J. Am. Chem. Soc. 122 (2000) 11232.
[10] Z.J. Chen, B.J. Lemon, S. Huang, D.J. Swartz, J.W. Peters, K.A. Bagley, Biochemistry 41 (2002) 2036.
[11] A. Volbeda, J.C. Fontecilla-Camps, Coord. Chem. Rev. 249 (2005) 1609.
[12] P.M. Vignais, Coord. Chem. Rev. 249 (2005) 1677.
[13] H. Reihlen, A. Gruhl, G. Hessling, Liebigs Ann. Chem. 472 (1929) 268.
[14] D. Seyferth, G.B. Womack, C.M. Archer, J.C. Dewan, Organometallics 8 (1989) 430.
[15] E.J. Lyon, I.P. Georgakaki, J.H. Reibenspies, M.Y. Darensbourg, Angew. Chem. Int. Ed. 38 (1999) 3178.
[16] M. Schmidt, S.M. Contakes, T.B. Rauchfuss, J. Am. Chem. Soc. 121 (1999) 9736.
[17] A. Le Cloirec, S.P. Best, S. Borg, S.C. Davies, D.J. Evans, D.L. Hughes, C.J. Pickett, Chem. Commun. (1999) 2285.
[18] J.D. Lawrence, H.X. Li, T.B. Rauchfuss, M. Benard, M.M. Rohmer, Angew. Chem. Int. Ed. 40 (2001) 1768.
[19] L.C. Song, Z.Y. Yang, H.Z. Bian, Q.M. Hu, Organometallics 23 (2004) 3082.
[20] E.J. Lyon, I.P. Georgakaki, J.H. Reibenspies, M.Y. Darensbourg, J. Am. Chem. Soc. 123 (2001) 3268.
[21] F. Gloaguen, J.D. Lawrence, M. Schmidt, S.R. Wilson, T.B. Rauchfuss, J. Am. Chem. Soc. 123 (2001) 12518.
[22] S.J. George, Z. Cui, M. Razavet, C.J. Pickett, Chem.-Eur. J. 8 (2002) 4037.
[23] M. Razavet, S.C. Davies, D.L. Hughes, C.J. Pickett, Chem. Commun. (2001) 847.
[24] M. Razavet, S.C. Davies, D.L. Hughes, J.E. Barclay, D.J. Evans, S.A. Fairhurst, X.M. Liu, C.J. Pickett, Dalton Trans. (2003) 586.
[25] G. Zampella, M. Bruschi, P. Fantucci, M. Razavet, C.J. Pickett, L. De Gioia, Chem.-Eur. J. 11 (2005) 509–533.
[26] L. De Gioia, Coord. Chem. Rev. 249 (2005) 1620.
[27] A. Paschos, A. Bauer, A. Zimmermann, E. Zehelein, A. Bock, J. Biol. Chem. 277 (2002) 49945.
[28] S. Reissmann, E. Hochleitner, H.F. Wang, A. Paschos, F. Lottspeich, R.S. Glass, A. Bock, Science 299 (2003) 1067.
[29] M.C. Posewitz, P.W. King, S.L. Smolinski, L.P. Zhang, M. Seibert, M.L. Ghirardi, J. Biol. Chem. 279 (2004) 25711.
[30] G.D. Cody, N.Z. Boctor, T.R. Filley, R.M. Hazen, J.H. Scott, A. Sharma, H.S. Yoder, Science 289 (2000) 1337.
[31] H.X. Li, T.B. Rauchfuss, J. Am. Chem. Soc. 124 (2002) 726.
[32] C.V. Popescu, E. Munck, J. Am. Chem. Soc. 121 (1999) 7877.
[33] A.S. Pereira, P. Tavares, I. Moura, J.J.G. Moura, B.H. Huynh, J. Am. Chem. Soc. 123 (2001) 2771.
[34] M.W.W. Adams, Biochim. Biophys. Acta 1020 (1990) 115.
[35] M. Razavet, S.J. Borg, S.J. George, S.P. Best, S.A. Fairhurst, C.J. Pickett, Chem. Commun. (2002) 700.
[36] I.P. Georgakaki, L.M. Thomson, E.J. Lyon, M.B. Hall, M.Y. Darensbourg, Coord. Chem. Rev. 238 (2003) 255.
[37] Z.X. Cao, M.B. Hall, J. Am. Chem. Soc. 123 (2001) 3734.
[38] Z.P. Liu, P. Hu, J. Am. Chem. Soc. 124 (2002) 5175.
[39] J.D. Lawrence, T.B. Rauchfuss, S.R. Wilson, Inorg. Chem. 41 (2002) 6193.
[40] C. Tard, X. Liu, D.L. Hughes, C.J. Pickett, Chem. Commun. (2005) 133–135.
[41] B.K. Burgess, D.J. Lowe, Chem. Rev. 96 (1996) 2983.
[42] D.L. Hughes, S.K. Ibrahim, H.M. Ali, C.J. Pickett, J. Chem. Soc., Chem. Commun. (1994) 425.
[43] G. Le Borgne, D. DGrandjean, R. Mathieu, R. Poilblanc, J. Organomet. Chem. 131 (1977) 429.
[44] X. Zhao, I.P. Georgakaki, M.L. Miller, J.C. Yarbrough, M.Y. Darensbourg, J. Am. Chem. Soc. 123 (2001) 9710.
[45] X. Zhao, I.P. Georgakaki, M.L. Miller, R. Mejia-Rodriguez, C.Y. Chiang, M.Y. Darensbourg, Inorg. Chem. 41 (2002) 3917.
[46] A. Farkas, L. Farkas, J. Yudkin, Proc. R. Soc. London (1934) 373.
[47] R. Cammack, V.M. Fernandez, E.C. Hatchikian, Meth. Enzymol. 243 (1994) 43.
[48] F. Gloaguen, J.D. Lawrence, T.B. Rauchfuss, M. Benard, M.M. Rohmer, Inorg. Chem. 41 (2002) 6573.
[49] J.L. Nehring, D.M. Heinekey, Inorg. Chem. 42 (2003) 4288.
[50] Y. Nicolet, A.L. de Lacey, X. Vernede, V.M. Fernandez, E.C. Hatchikian, J.C. Fontecilla-Camps, J. Am. Chem. Soc. 123 (2001) 1596.
[51] S.J. Borg, T. Behrsing, S.P. Best, M. Razavet, X.M. Liu, C.J. Pickett, J. Am. Chem. Soc. 126 (2004) 16988.
[52] R. Mejia-Rodriguez, D.S. Chong, J.H. Reibenspies, M.P. Soriaga, M.Y. Darensbourg, J. Am. Chem. Soc. 126 (2004) 12004.
[53] L. Sun, B. Akermark, S. Ott, Coord. Chem. Rev. 249 (2005) 1653.
[54] M.D. Westmeyer, T.B. Rauchfuss, A.K. Verma, Inorg. Chem. 35 (1996) 7140.
[55] J.D. Lawrence, H.X. Li, T.B. Rauchfuss, Chem. Commun. (2001) 1482.
[56] T.B. Liu, M. Wang, Z. Shi, H.G. Cui, W.B. Dong, J.S. Chen, B. Akermark, L.C. Sun, Chem.-Eur. J. 10 (2004) 4474.
[57] C. Kolomyjec, J. Whelan, B. Bosnich, Inorg. Chem. 22 (1983) 2343.
[58] C.J. Pickett, K.S. Ryder, J. Chem. Soc., Dalton Trans. (1994) 2181.
[59] C. Tard, X.M. Liu, S.K. Ibrahim, M. Bruschi, L. De Gioia, S.C. Davies, X. Yang, L.S. Wang, G. Sawers, C.J. Pickett, Nature 434 (2005) 610.
[60] J. Talarmin, F. Gloaguen, P. Schollhammer, Coord. Chem. Rev. 249 (2005) 1664.