Structural variability of the active site of Fe-only hydrogenase and its hydrogenated forms

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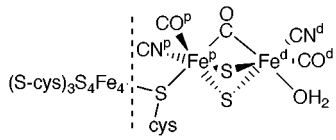
Received (in Cambridge, UK) 12th May 1999, Accepted 21st July 1999

Density functional calculations show that the (cys-S)-(CO)(CN)FeS_2(μ-CO)Fe(CO)(CN) active site of the Fe-only hydrogenase from *Clostridium pasteurianum* is redox ambivalent and stereochemically flexible at the CO and S bridges, and at the Fe atoms, and with bound hydrogen: the fundamentals of probable mechanisms are revealed.

The hydrogenase enzymes, which catalyse the reaction $H_2 \rightleftharpoons 2H^+ + 2e^-$, occur as the FeNi-enzymes which mainly oxidise H_2,[1–3] and as the Fe-only enzymes which mainly reduce hydrons H^+.[1,4,5] Crystal structures of both types have been reported recently,[6–12] and reveal the presence of biologically unusual CO and CN ligands at the active sites. In particular, the active site of Fe-hydrogenase from *Clostridium pasteurianum* (CpI)[9] contains an Fe_2 site, **I**, in which two Fe atoms are each coordinated by one CO and one CN^- ligand, and bridged by two S atoms and one CO ligand, as shown in Fig. 1: **I** is linked by bridging cysteine to a cubanoid Fe_4S_4(S-cys)$_4$ cluster at the end of an evident electron transfer pathway. Assignment and differentiation of the CO and CN^- ligands in **I** is from the IR data[13] coupled with the occurrence of two hydrogen bonds from surrounding protein.[14]



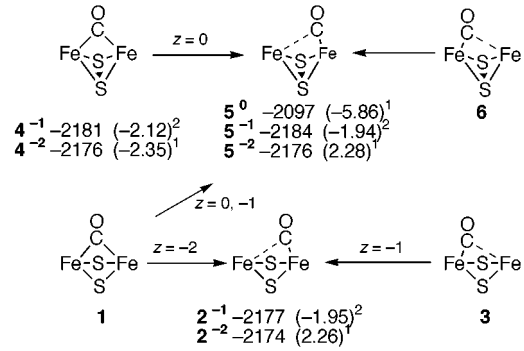
I

Fig. 1 The Fe_2 site **I** as revealed crystallographically for the Fe hydrogenase from *Clostridium pasteurianum*.[9] The Fe atoms and their ligands are labelled as proximal (Fe^p) and distal (Fe^d) to the Fe_4S_4 cluster.

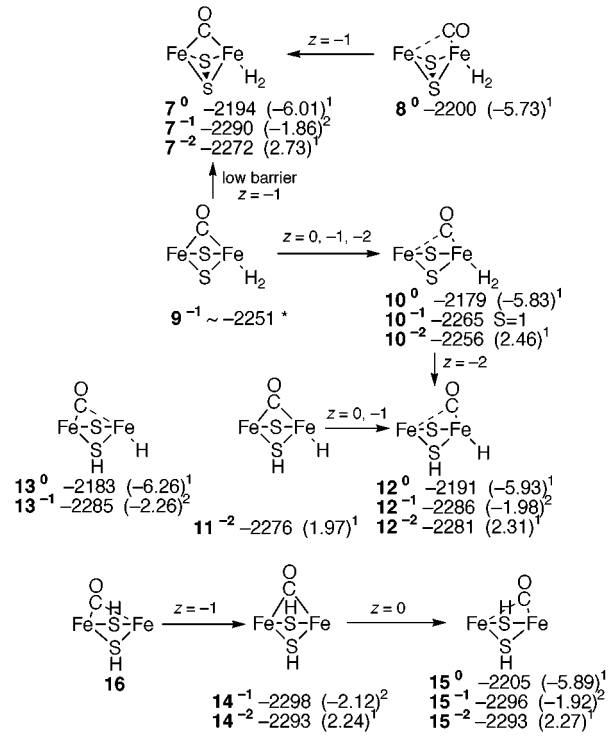
The Fe_2 site **I** is considered to control hydron reduction, the simplest of chemical reactions, but **I** is unprecedented in chemistry. I have investigated the key questions—redox states, stereochemical flexibility, electronic structure, binding of H^+, H and H_2, and mechanism—by density functional calculations† on $[(CH_3S)(CO)(CN)FeS_2(\mu\text{-}CO)Fe(CO)(CN)]^z$, **II**, in which CH_3S^- replaces the cysteine at Fe^p.

Molecule **II** has two low-energy stereochemical variables: (1) the bridging CO ligand can easily swing like a gate between Fe^p and Fe^d; (2) the two bridging S atoms can be separate (at *ca.* 3.3 Å, symbolised S/S), or bonded (S–S, at *ca.* 2.1 Å). Both of these variables are evident in the results shown in Scheme 1, which shows the energies and barrierless transformations of six isomers at three possible redox levels specified by the total charge *z*. The small energy associated with shifts of the CO bridge is evident in the energies of **4** and **5**: $E(\mathbf{4}^{-2}) = E(\mathbf{5}^{-2})$; $E(\mathbf{4}^{-1}) = E(\mathbf{5}^{-1}) + 3$ kcal mol^{-1}; $\mathbf{4}^0$ transforms to $\mathbf{5}^0$ without a barrier. The CO gate shifts left to right for both the S/S (**3** → **2**) and S–S (**6** → **5**) structures, because Fe^d would otherwise be only four-coordinate. Note the small energy differences between isomers with and without S–S bonding: $\mathbf{5}^{-1}$ with an S–S bond is within 7 kcal mol^{-1} of $\mathbf{2}^{-1}$, while for charge –2 the energy difference between **5** and **2** is only 2 kcal mol^{-1}. There are substantial stereochemical changes at Fe^p and Fe^d concomitant with changes in the bridge.

Similar results are found for a model with OH_2 bound to Fe^d. In particular, isomer **1–OH$_2$** as observed in the crystal[9] transforms to isomer **5–OH$_2$** with S–S bonding. There is good agreement between the other bond distances observed and calculated (in parentheses) for **5–OH$_2$**: Fe–Fe 2.62 (2.63); Fe–S



Scheme 1 Relationships between six isomers of $[(CH_3S)(CO)(CN)FeS_2$-(CO)Fe(CO)(CN)]^z, **II**. In these diagrams the CH_3S, terminal CO and CN ligands are omitted; superscripts are the charges *z*. The numbers listed for each minimum are the total energy (kcal mol^{-1}, relative to free atoms) and the (energy/eV)^{population} of the HOMO. Arrows identify barrierless exergonic transformations.



Scheme 2 Isomers of **II** plus two H atoms: diagrams are simplified and information summarised as for Scheme 1. **7**, **8**, **9** and **10** have Fe–H_2 coordination; **11**, **12** and **13** contain SH and Fe–H; **14**, **15** and **16** have $(SH)_2$. * Close-lying electronic states.

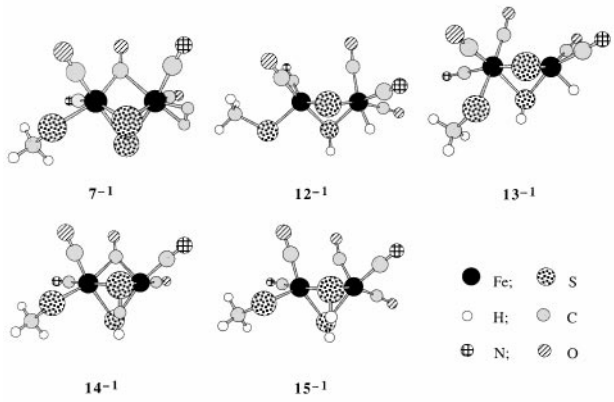


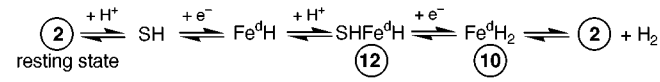
Fig. 2 Optimised structures for five isomers containing 2H, with similar energies but very different bonding patterns, and variations in the coordination stereochemistry at Fe.

2.32–2.34 (2.31–2.39); Fe–SCH_2 2.38 (2.38); Fe-(μ-CO) 2.04–2.10 (1.95–2.03) Å.

What about the redox levels? For $\mathbf{2}^{-1}$ with an $(S^{2-})_2$ bridge the formal oxidation states are $(Fe^{III})_2$ and the doubly occupied HOMO is at −1.95 eV. Reduction to $\mathbf{2}^{-2}$ raises the HOMO to positive energy (Scheme 1). In contrast, $\mathbf{5}^{-1}$ with an $(S_2)^{2-}$ bridge is formally $(Fe^{II})_2$ with the HOMO at −1.95 eV, and $\mathbf{5}^0$ (Fe^{II}, Fe^{III}) has its HOMO at a normal energy of −5.86 eV. In this context it is significant that the Fe_2 hydrogenase from *Desulfovibrio desulfuricans* has a dithiolate bridge $^-SCH_2CH_2CH_2S^-$,[12] and the NiFe hydrogenases have bis-cysteine bridges $(RS^-)_2$,[6–8,10,11] all of which involve redox levels like **5**, not **2**. Extra electron density in the bridging region of CpI[9] could be a dithiolate bridge,[18] or partial occurrence of a S_2^{2-} bridge.

What happens when the active site binds H^+ (at S), H (at S and/or Fe), or H_2 (at Fe)? Scheme 2 displays structures, energies and electronic states of isomers for the model **II** + 2H. Dihydrogen binds to Fe^d, although weakly. The least stable isomer is **9** (*i.e.* the crystal structure with H_2 bound at Fe^d in place of OH_2), which is stabilised by a CO gate shift right (to **10**). In its reduced state $\mathbf{9}^{-2}$ undergoes a mechanistically significant barrierless transfer of one H atom from H_2–Fe to form SH (→ $\mathbf{12}^{-2}$). There is a low barrier for the exergonic S–S bond formation in $\mathbf{9}^{-1}$ (→ $\mathbf{7}^{-1}$). A significant result is that chemically different isomers in Scheme 2 have very similar energies: examples are: $\mathbf{15}^{-1}$ −2296 [$(SH)_2$, CO-right], $\mathbf{14}^{-1}$ −2293 [$(SH)_2$, CO-sym], $\mathbf{7}^{-1}$ −2290 [S–S, Fe–H_2, CO-sym], $\mathbf{12}^{-1}$ −2286 [SH, Fe–H, CO-right], $\mathbf{13}^{-1}$ −2285 [SH, Fe–H, CO-left] kcal mol^{-1}. The calculated geometries of these are presented in Fig. 2, which also emphasises the stereochemical flexibility at Fe^p and Fe^d.

The main conclusion is that the unusual active site of this Fe-only hydrogenase possesses a relatively flat potential energy surface for geometrical change at Fe, CO, S, and bound H. The species in Scheme 2, and others not shown, can be combined in various ways as mechanisms to be calculated in further detail. It is likely that the mechanism involves the following fundamentals:



This research is supported by the Australian Research Council and the University of NSW.

Notes and references

† BLYP functional, numerical basis sets, spin restricted and unrestricted, in the program DMol, as described previously.[15–17]

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Communication 9/03803E