Functionally Relevant Interplay between the Fe(4)S(4) Cluster and CN(-) Ligands in the Active Site of [FeFe]-Hydrogenases.

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Functionally relevant interplay between the Fe_4S_4 cluster and CN^- ligands in the active site of [FeFe]-hydrogenases.

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Molecular hydrogen evolution from protons and electrons is a key reaction in microbial redox metabolism.[1] [FeFe]-hydrogenases, which are very efficient hydrogen evolving enzymes, are able to catalyze H_2 production at very low overpotentials and mild conditions (–0.4 V vs. NHE at neutral pH), and with a turnover number as large as 6000 s^{-1}.[2] Due to their remarkable efficiency, [FeFe]-hydrogenases represent an ideal paradigm for the development of biomimetic electrocatalysts for H_2 production from acidic aqueous solutions. The [FeFe]-hydrogenases active site contains an Fe_6S_6 complex (referred to as H-cluster), which is composed by a classical Fe_4S_4 cubane cluster, covalently linked by a cysteine sulfur atom to a Fe_2S_2 subcluster (referred to as $[2Fe]_H$).[3,4] The Fe atoms of the binuclear subcluster are coordinated by three CO and two CN^- ligands, and by a bidentate chelating ligand of formula $^-SCH_2ZCH_2S^-$, where Z has been proposed to correspond to a CH_2 group, a NH group or an oxygen atom (see the group marked with an asterisk in Fig. 1). Several evidences suggest that H_2 formation takes place at the Fe_d atom of the $[2Fe]_H$ cluster (atom labels in Fig. 1).[5-8]

The disclosure of key relationships between the structure of the enzyme active site and its reactivity has been very nicely complemented in recent years by the investigation of coordination compounds structurally related to the [FeFe]-hydrogenase active site.[9-12] However, the catalytic efficiency of available bioinspired catalysts is still much lower then that of the enzyme. This could be due, among other factors, to two reasons: First, most of the bioinspired catalysts are binuclear complexes, i.e. they do not include the Fe_4S_4 subcluster. This difference, together with the lack of the protein matrix, can lead to a shift of the redox potential of the synthetic catalysts to more negative voltages relative to the enzyme. In fact, the only existing synthetic Fe_6S_6 complex (**1**, $[Fe_4S_4(L)_3\{Fe_2(CH_3C(CH_2S)_3)(CO)_5\}]^{2-}$, L = 1,3,5-tris-(4,6-di-methyl-3-mercaptophenylthio)-2,4,6-tris-(p-tolyl-thio)-benzene) closely resembling the structure of the H-cluster (but differing from it by replacement of CN ligands with CO groups),[13] has a reduction potential 0.3 V less negative then the corresponding binuclear analogue.[14] Second, CN^- groups, which are strictly conserved among all [FeFe]-hydrogenases, are usually replaced by other ligands in synthetic catalysts, mainly because CN^- groups compete with Fe atoms for proton binding in the absence of the protein matrix. In this context, it is worth noting that while some functional roles of CN^- ligands in the enzyme cofactor has been already highlighted, e.g. to increase the basicity of the $[2Fe]_H$ core,[15] as well as to "freeze" the binuclear cluster in a functionally competent inverted pyramidal structure[16] – the effects of the simultaneous presence of the Fe_4S_4 cluster and CN^- ligands on functional properties of the enzyme have not been explored.

Prompted by the above observations, with the aim of disclosing a possible interplay between the Fe_4S_4 cluster and CN^- ligands in the active site of [FeFe]-hydrogenases, as well as its functional relevance, we have carried out QM/MM calculations on the wild-type [FeFe]-hydrogenase from *Desulfovibrio desulfuricans* (PDB code: 1HFE), and on a variant of the enzyme in which the cyanides in the H-cluster have been replaced by CO. In both cases, the reduced ($\mathbf{H}_{red}$ and $\mathbf{(2CO)H}_{red}$) and the oxidized protein forms ($\mathbf{H}_{ox}$ and $\mathbf{(2CO)H}_{ox}$) – which are catalytically active in the wild-type case – were studied (Fig. 1).

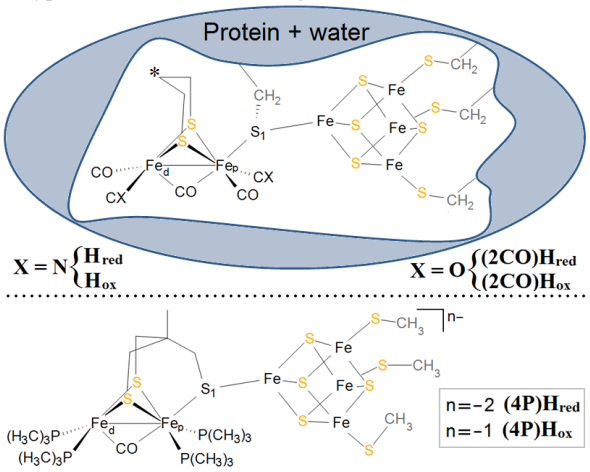

Figure 1. QM/MM models of the enzyme forms $\mathbf{H}_{red}$, $\mathbf{H}_{ox}$, $\mathbf{(2CO)H}_{red}$, $\mathbf{(2CO)H}_{ox}$, and scheme of the purely QM models $\mathbf{(4P)H}_{red}$ and $\mathbf{(4P)H}_{ox}$

QM/MM calculations show that in the $\mathbf{H}_{red}$ form of the protein, which corresponds to a Fe(I)Fe(I), 2Fe(III)2Fe(II) redox state, the HOMO (highest occupied molecular orbital), which influences protonation regiochemistry, is localized on the Fe_d atom of the $[2Fe]_H$ cluster, whereas the LUMO (lowest unoccupied molecular orbital) is only slightly higher in energy and confined to the Fe_4S_4 clusters. The functional relevance of a small energy gap between frontier orbitals in $\mathbf{H}_{red}$ was previously highlighted,[16,17] showing how subtle modification of the environment (such as protonation of the bidentate ligand or a nearby amino acid) can invert the energies of the frontier orbitals, triggering electron transfer between the two subclusters.

The replacement of CN^- with CO ligands in $\mathbf{H}_{red}$ leads to a significant drop in energy of the orbitals localized on the $[2Fe]_H$ cluster (Fig. 2). As a consequence, the highest occupied molecular orbitals become localized on the Fe_4S_4 cluster, affecting protonation regiochemistry (which in $\mathbf{(2CO)H}_{red}$ would take place on the Fe_4S_4 cluster) and switching off the electronic communication between the two subclusters.

The reorganization of the electronic structure of the H-cluster upon CN^-/CO substitution turns out to be even more evident in

the $\mathbf{H_{ox}}$ enzyme form, in which the active site is one-electron oxidized with respect to $\mathbf{H_{red}}$ and attains the Fe(II)Fe(I), 2Fe(III)2Fe(II) state. While in the wild-type enzyme, one-electron oxidation of the H-cluster takes place on the $[2Fe]_H$ subcluster, in the oxidation of $\mathbf{(2CO)H_{red}}$ the electron is removed from the Fe_4S_4 cluster (see Fig. 3), which therefore reaches a 3Fe(III)Fe(II) redox state, while the $[2Fe]_H$ cluster maintains the Fe(I)Fe(I) redox state.

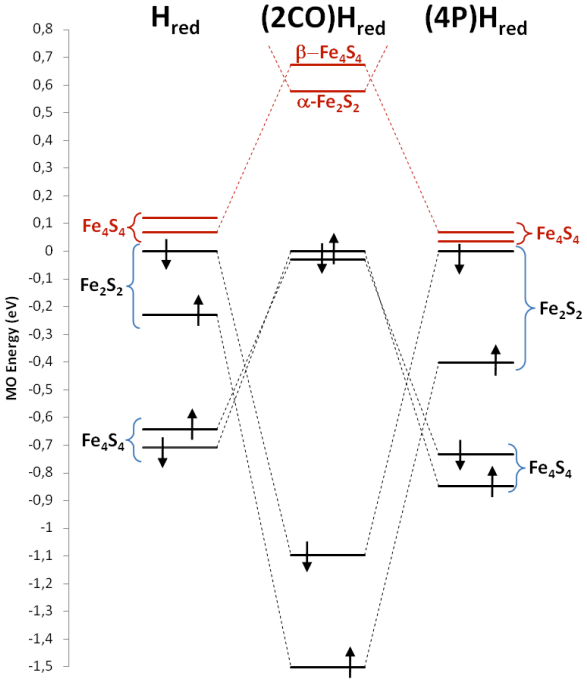

Figure 2. Frontier molecular orbital (FMO) relative energies (in eV) with respect to the HOMO for the reduced forms of the systems investigated in this paper. Each FMO is labeled according to whether it belongs to the Fe_4S_4-SR or the Fe_2S_2 fragment. This assignment has been made according to the contribution of the atomic basis coefficients to a given MO with a 50% criterion for each fragment. Dashed lines connect FMOs of two different systems that belong to the same fragment.

In summary, the QM/MM results disclose a functionally relevant interplay between CN^- ligands and the Fe_4S_4 cluster in the active site of [FeFe]-hydrogenases. In particular, the CN^- ligands in the H-cluster play a pivotal role to maintain frontier orbitals close in energy and localized on different subclusters, allowing facile electron transfer between the two subcluster, and localize the HOMO in $\mathbf{H_{red}}$ on the $[2Fe]_H$ subcluster, allowing proton binding to take place on the binuclear site of the H-cluster.

The QM/MM results also suggest that the presence of CN^- ligands could be crucial for the design and synthesis of *functional* biomimetic Fe_6S_6 complexes. However, as stated in the introduction, the presence of CN^- ligands in synthetic complexes is not optimal because they can compete with Fe atoms for proton binding. Therefore, we have searched for a suitable combination of ligands, not including CN^-, that can replace the CO group in complex **1** and lead to a species characterized by electronic features similar to the H-cluster. In this context, phosphines, which have already proved to be convenient for the synthesis of analogues of [FeFe]-hydrogenases,[10] are a natural choice. Given the dicyanide nature of the wild-type H-cluster, diphosphine analogs of **1** were considered first; even though the inclusion of two $P(CH_3)_3$ groups in place of two carbonyls somehow elevates the redox properties of the binuclear site (data not shown), the presence of four $P(CH_3)_3$ groups ($\mathbf{(4P)H_{red}}$ and $\mathbf{(4P)H_{ox}}$, Fig. 1) proved sufficient to finely reproduce the redox and electronic features of the enzyme cofactor, with the highest occupied and lowest unoccupied orbitals localized on the Fe_2S_2 and the Fe_4S_4 cluster, respectively (see Fig. 2). Electron communication between the two subclusters is also restored, as indicated by the small HOMO–LUMO energy gap. Moreover, in the one-electron oxidation of $\mathbf{(4P)H_{red}}$ the electron is removed from the Fe_2S_2 subcluster, as observed in the wild-type H-cluster (see Fig. 3). Furthermore, in the reduced complex, the HOMO, suitable for H^+ binding, is localized on the Fe_2S_2 cluster, again as observed in the wild-type H-cluster.

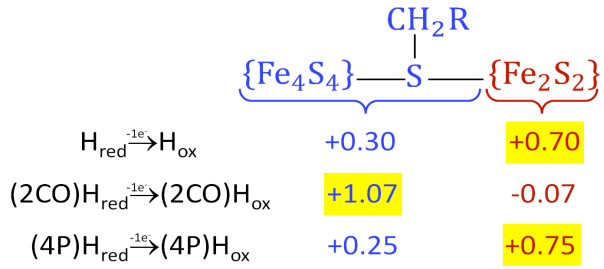

Figure 3. Change in the Mulliken populations of Fe_4S_4-SR and Fe_2S_2 fragments, upon one-electron oxidation of the various Fe_6S_6 assemblies here investigated. The highest values (highlighted in yellow) indicate on which fragment the oxidation process takes place.

In conclusion, while the role of the cyanide ligands to increase the basicity of the H-cluster had been already highlighted, our QM and QM/MM results have disclosed a more subtle, but crucial role played by the two CN^- ligands in the active site of [FeFe]-hydrogenases. In fact, cyanide groups fine-tune the electronic and redox properties of the active site, affecting both protonation regiochemistry and electron transfer between the two subclusters of the H-cluster. Therefore, in the design of bioinspired Fe_6S_6 synthetic complexes their important role should be taken into account. In this respect, we have shown that the replacement of four CO ligands in the synthetic complex **1** with phosphine ligands may restore the electronic and redox features of the wild-type H-cluster.

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Supporting Information Available: Detailed computational methods for QM and QM/MM calculations. Cartesian coordinates, Mulliken atomic charges and spin populations.

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Abstract:

[FeFe]-hydrogenases are highly efficient H_2-evolving metalloenzymes that include cyanides and carbonyls in the active site. The latter is a Fe_6S_6 cluster – the so-called H-cluster – that can be subdivided in a binuclear portion carrying the CO and CN^- groups, and a tetranuclear subcluster. The fundamental role of cyanide ligands for increasing the basicity of the H-cluster has been already highlighted. Now, a more subtle but crucial role played by the two CN^- ligands in the active site of [FeFe]-hydrogenases is disclosed. In fact, QM/MM calculations on all-atom models of the enzyme from *D. desulfuricans* show that the cyanide groups fine-tune the electronic and redox properties of the active site, affecting both protonation regiochemistry and electron transfer between the two subclusters of the H-cluster. Despite the crucial role of cyanides in the protein active site, the currently available bio-inspired electrocatalysts generally lack CN^- groups, to avoid competition between the latter and the catalytic metal centers for proton binding. In this respect, we show that a targeted inclusion of phosphine ligands in hexanuclear biomimetic clusters may restore the electronic and redox features of the wild-type H-cluster.

Graphical TOC: