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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 under mild conditions (−0.4 V vs NHE at neutral pH) and with turnover numbers as large as 6000 s^{-1}.[2] Because of their remarkable efficiency, [FeFe]-hydrogenases represent an ideal paradigm for the development of biomimetic electrocatalysts for H_2 production from acidic aqueous solutions. The active site of [FeFe]-hydrogenases contains an Fe_6S_6 complex (known as the H-cluster) composed of a classical Fe_4S_4 cubane cluster covalently linked by a cysteine sulfur atom to an Fe_2S_2 subcluster (designated 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 having the formula $^-SCH_2ZCH_2S^-$, where Z has been proposed to be a CH_2 group, an NH group, or an oxygen atom (see the group marked with an asterisk in Figure 1). Several pieces of evidence suggest that H_2 formation takes place at the Fe_d atom of the $[2Fe]_H$ cluster (atom labels are shown in Figure 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 than that of the enzyme. This could be due to two factors (among others). 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-dimethyl-3-mercaptophenylthio)-2,4,6-tris(*p*-tolylthio)benzene) that closely resembles the structure of the H-cluster (but differs from it by replacement of CN^- ligands with CO groups)[13] has a reduction potential 0.3 V less negative than 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 have been highlighted previously (e.g., increasing the basicity of the $[2Fe]_H$ core[15] as well as "freezing" the binuclear cluster in a functionally competent inverted pyramidal structure[16]), the effects of the simultaneous presence of the Fe_4S_4 cluster and the CN^- ligands on the functional properties of the enzyme have not been explored.

Prompted by the above observations and with the aim of disclosing the possible existence and functional relevance of an interplay between the Fe_4S_4 cluster and the CN^- ligands in the active site of [FeFe]-hydrogenases, we carried out quantum mechanical (QM)/molecular mechanics (MM) calculations on the wild-type [FeFe]-hydrogenase from *Desulfovibrio desulfuricans* (PDB entry 1HFE) and a variant of the enzyme in which the cyanides in the H-cluster were replaced by CO; in both cases, the reduced [$\mathbf{H_{red}}$ and $\mathbf{(2CO)H_{red}}$] and oxidized [$\mathbf{H_{ox}}$ and $\mathbf{(2CO)H_{ox}}$] protein forms, which are catalytically active in the wild-type case, were studied (Figure 1).

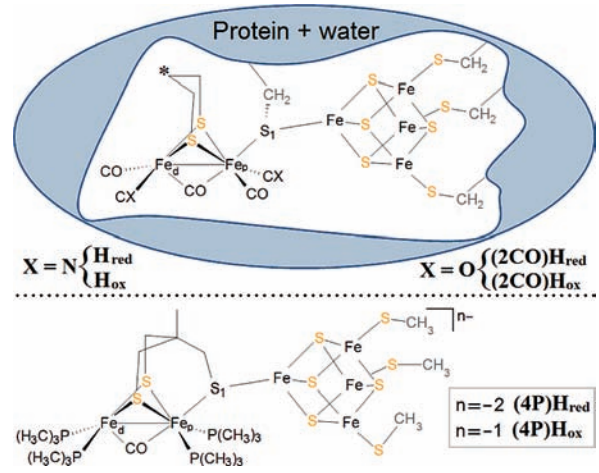

Figure 1. (top) QM/MM models of the enzyme forms $\mathbf{H_{red}}$, $\mathbf{H_{ox}}$, $\mathbf{(2CO)H_{red}}$, and $\mathbf{(2CO)H_{ox}}$ and (bottom) the purely QM models $\mathbf{(4P)H_{red}}$ and $\mathbf{(4P)H_{ox}}$.

The QM/MM calculations show that in the $\mathbf{H_{red}}$ form of the protein, which corresponds to an Fe(I)Fe(I)−2Fe(III)2Fe(II) redox state, the highest occupied molecular orbital (HOMO), which influences the protonation regiochemistry, is localized on the Fe_d atom of the $[2Fe]_H$ cluster, whereas the lowest unoccupied molecular orbital (LUMO) is only slightly higher in energy and confined to the Fe_4S_4 cluster. The functional relevance of a small energy gap between the frontier orbitals in $\mathbf{H_{red}}$ was highlighted previously,[16,17] showing how a 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 the CN^- ligands with CO in $\mathbf{H_{red}}$ leads to a significant drop in the energy of the orbitals localized on the $[2Fe]_H$ cluster (Figure 2). As a consequence, the HOMO becomes localized on the Fe_4S_4 cluster, affecting the 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.

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

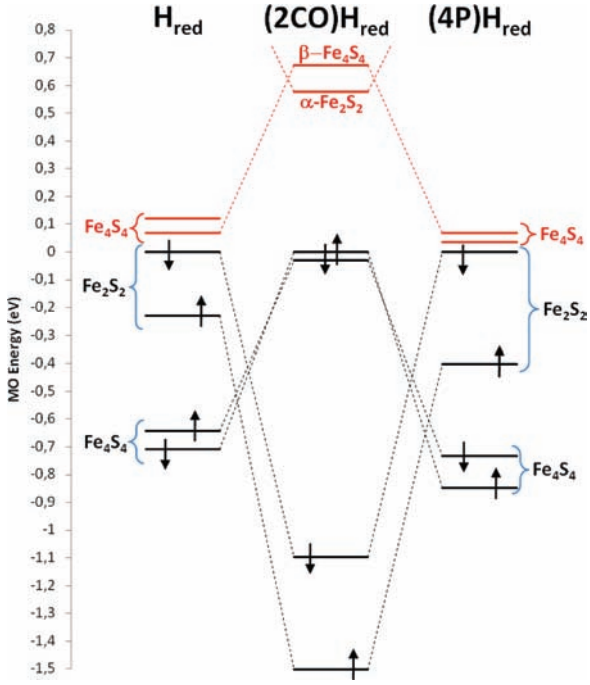

Figure 2. Frontier molecular orbital (FMO) relative energies (in eV) with respect to the HOMO for the reduced forms of the systems investigated in this work. Each FMO is labeled according to whether it belongs to the Fe_4S_4−SR or Fe_2S_2 fragment. This assignment was made according to the contribution 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 the 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 in maintaining the frontier orbitals close in energy and localized on different subclusters, allowing facile electron transfer between the two subclusters and localizing the HOMO in $\mathbf{H_{red}}$ on the $[2Fe]_H$ subcluster, which causes proton binding to take place on the binuclear site of the H-cluster.

The QM/MM results also suggest that the presence of the 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 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 those of 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 analogues of **1** were considered first; even though the inclusion of two $P(CH_3)_3$ groups in place of the two carbonyls somehow improved the redox properties of the binuclear site from a biomimetic perspective (data not shown), the presence of four $P(CH_3)_3$ groups [$\mathbf{(4P)H_{red}}$ and $\mathbf{(4P)H_{ox}}$; Figure 1] proved sufficient to finely reproduce the redox and electronic features of the enzyme cofactor, with the HOMO and LUMO localized on the Fe_2S_2 and Fe_4S_4 clusters, respectively (see Figure 2). Electron communication between the two subclusters was 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 Figure 3). Furthermore, in the reduced complex, the HOMO, which is suitable for H^+ binding, is localized on the Fe_2S_2 cluster, again as observed in the wild-type H-cluster.

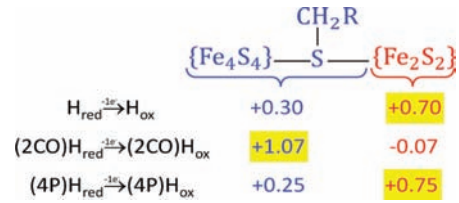

Figure 3. Change in the Mulliken charges of the Fe_4S_4−SR and Fe_2S_2 fragments upon one-electron oxidation of the various Fe_6S_6 assemblies investigated here. The highest values (highlighted in yellow) indicate the fragments in which the oxidation process takes place.

In conclusion, while the role of the cyanide ligands in increasing the basicity of the H-cluster had been highlighted previously, 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, the cyanide groups fine-tune the electronic and redox properties of the active site, affecting both the 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 targeted 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, along with Cartesian coordinates, Mulliken atomic charges, and spin populations. This material is available free of charge via the Internet at http://pubs.acs.org.

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