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Towards biomimetic models of the reduced [FeFe]-hydrogenase that preserve the key structural features of the enzyme active site; a DFT investigation

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ABSTRACT

[FeFe]-hydrogenases are the most efficient biological catalysts available for the H_2 evolution reaction. Their active site – the H-cluster – features a diiron subsite which has the peculiar characteristic of bearing cyanide groups hydrogen-bonded to the apoprotein as well as carbonyl ligands. Notably, one of the CO ligands is disposed in bridging position between the metal centers. This allows one of the Fe ions to retain a square pyramidal coordination – which determines the assumption of the so-called "rotated structure" – with a vacant coordination site in *trans* to the μ-CO group, ready to bind protons when the active site is in the $Fe^{I}Fe^{I}$ state. Many $Fe^{I}Fe^{I}$ biomimetic models have been synthesized and characterized so far, but most of them fail to reproduce the orientation of the diatomic ligands that is observed in the enzyme active site.

In the present contribution we carried out a density functional theory investigation, with the aim of evaluating whether the establishment of hydrogen bonding at the level of cyanides is sufficient to favor rotation of ligands around one of the Fe centers, in analogy with the reduced H-cluster. To this end, we carried out an investigation of the potential energy surface of an isolated Fe_2S_2 model bearing CN and CO groups, as well as of the supramolecular complex formed by the diiron model and a porphyrin derivative hydrogen-bonded to the former. As far as the isolated Fe_2S_2 species are concerned, the sole μ-CO models individuated in the course of our potential energy surface scans are the ones in which both cyanides are bound to same iron center, while no CO-bridged minima could be found in the case of models having one CN group bound to each of the metal ions. The latter represents a major difference with respect to the coordination geometry of the reduced diiron subcluster in the enzyme. However, perturbation of the diiron model by a porphyrin ring designed to donate hydrogen bonds to the cyanide groups – thus, at least partially, reproducing the network of H-bond between the H-cluster and the apoprotein in

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[FeFe]-hydrogenases – significantly changes the picture in this regard. Therefore, possible strategies to modulate the disposition of ligands around the metal centers of biomimetic [FeFe]-hydrogenases models are discussed in light of computed geometries and relative stabilities of the supramolecular complexes.

1. Introduction

Hydrogenases are enzymes expressed by microorganisms that are able to metabolize molecular hydrogen and/or to evolve H_2 from protons [1]. They are iron-containing metalloproteins that can be subdivided in three main classes: the iron-sulfur cluster free hydrogenases, which feature a monoiron catalytic site; the [NiFe]-hydrogenases, characterized by the presence of a catalytically relevant nickel atom; and the [FeFe]-hydrogenases, which feature the highest catalytic efficiency among the three classes so far characterized [2]. Such high activity depends on the stereoelectronic features of the [FeFe]-hydrogenases active site, which is generally referred to as the H-cluster. X-ray structures of [FeFe]-hydrogenases, as well as spectroscopic studies, revealed the very peculiar structure of the H-cluster [3,4], which can be described as a classical [4Fe4S] cubane cluster (referred to as $[4Fe4S]_H$) bridged by a cysteine residue to a diiron cluster, referred to as the $[2Fe]_H$ subcluster (Fig. 1). The iron atom of the $[2Fe]_H$ subcluster proximal to the $[4Fe4S]_H$ cluster (usually referred to as Fe_p) is coordinated by one CN^- and one CO ligand, and is bridged via one CO and a dithiomethylamine (dtma) ligand to the distal Fe atom (referred to as Fe_d). The Fe_d atom of the $[2Fe]_H$ subcluster is also coordinated by one CO and one CN^- ligand but features a vacant coordination position, which is the most likely site where substrates (dihydrogen and/or protons) and reversible inhibitors (CO) bind. Two catalytically competent redox states of the $[2Fe]_H$ subcluster have been extensively characterized spectroscopically. The reduced form of the enzyme, usually referred to as the H_{red} state, is EPR silent and corresponds to a Fe^IFe^I species. The partially oxidized form of [FeFe]-hydrogenases (H_{ox}) is paramagnetic and corresponds to a $Fe^{II}Fe^I$ redox state.

The disclosure of the structural features of the [FeFe]-hydrogenase active site paved the way to the investigation of plausible reaction pathways for enzymatic H_2 evolution and oxidation. In particular, experimental and computational results are consistent with the following scenario (Fig. 2): one

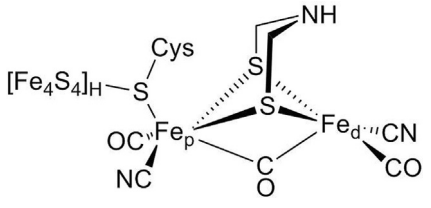

Fig. 1 – Schematic structure of the H-cluster found in the active site of [FeFe]-hydrogenases.

electron reduction and protonation of the H_{ox} form should yield a transient Fe^IFe^I species featuring protonated dtma. Then, proton transfer takes place from the ammonium group of dtma to the vacant coordination site on Fe_d [5,6]. The resulting $Fe^{II}Fe^{II}$ species, which features a terminal hydride ligand, is thought to undergo further protonation of the amine group in the pendant and one-electron reduction, yielding a mixed-valence $Fe^{II}Fe^I$ intermediate featuring transient dihydrogen coordination. H_2 detachment from Fe_d brings the H-cluster back to the initial $Fe^{II}Fe^I$ redox state. However, even if there is quite a good agreement about the general picture of the catalytic cycle, some ambiguities still exist. One of the still open issues concerns the structural features of the $[2Fe]_H$ subcluster in the H_{red} redox state. More specifically, even if several simple synthetic diiron dithiolate complexes with a composition strongly related to the structure of the $[2Fe]_H$ subcluster have been synthesized, most of the Fe^IFe^I complexes characterized to date fail to reproduce the orientation of the diatomic ligands that is observed in the enzyme active site. In particular, Fe^IFe^I synthetic complexes are usually characterized by an eclipsed geometry of the ligands coordinated to the metal ions (Fig. 3), which has never been observed in the catalytic site of [FeFe]-hydrogenases. Instead, the FeFe subcluster in the enzyme is thought to be characterized by a "rotated structure" of the ligands coordinated to Fe_d, which exhibits an essentially square pyramidal coordination with a vacant coordination site in *trans* to the μ-CO group.

Remarkably, the structural difference between the $[2Fe]_H$ subcluster and model complexes might be related to the poor catalytic properties of synthetic diiron complexes. In fact, experimental and computational studies led to propose that the peculiar structure of the $[2Fe]_H$ subcluster in the H_{red} state could be due to the disposition of iron ligands and also to specific interactions between the cyanide nitrogen atoms and the peptide chain. In particular, DFT calculations were carried out to evaluate how changes in the first coordination sphere of synthetic compounds can affect the structural features of the complex [7], suggesting that the combination of a sterically demanding dithiolate bridge with the asymmetric presence of strong donor ligands could be a viable strategy to obtain synthetic diiron complexes with structural properties similar to the $[2Fe]_H$ active site. More recently, it was experimentally shown that a synergy of crystal packing effects and agostic interactions between the dithiolate bridge and an iron center is able to stabilize the rotated conformation in Fe^IFe^I biomimetic models [8,9]. However, the most "natural" way to reproduce the key environmental factors present in the enzyme active site would imply the design of synthetic complexes in which the position of hydrogen bond donors is similar to that observed in the protein. Prompted by