8-5-2010

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Recommended Citation

Dogaru, Daniela; Motiu, Stefan; and Gogonea, Valentin, "Residue Mutations in [Fe-Fe]-Hydrogenase Impedes O 2 Binding: A QM/MM Investigation" (2010). Chemistry Faculty Publications. 328.  
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Residue Mutations in [Fe–Fe]-Hydrogenase Impedes $O_2$ Binding: A QM/MM Investigation

DANIELA DOGARU, STEFAN MOTIU, VALENTIN GOGONEA
**Introduction**

Fe–Fe-hydrogenases are enzymes that catalyze the reversible reduction of protons to molecular hydrogen (\(2H^+ + 2e^- \rightarrow H_2\)) in anaerobic media [1, 2], and are considered one of the oldest enzymes in nature [3]. The eventual elucidation of the catalytic mechanism of hydrogen synthesis may avail researchers produce clean hydrogen fuel, using certain prokaryotes and eukaryotes [4–13].

The active site of hydrogenase, the H-cluster (Scheme 1) is composed of two iron atoms (Fe_p–Fe_d, i.e., proximal and distal iron). The di-iron atoms are coordinated by endogenous ligands, i.e., two cyanides, two terminal carboxyls, and a bridging carbonyl (CO_b). Also, 1,3-di(thiomethyl)-amine (DTMA) and propanedithiolate (PDT) are bidentate ligands of the di-iron subsite [14–16]. A cubane cluster, [4Fe–4S] (which also belongs to the H-cluster), is bonded to S_γ of Cys^{382}, while the former (S_γ) is bound to Fe_p of the H-cluster.

Previous density functional theory (DFT) as well as hybrid quantum mechanics/molecular mechanics (QM/MM) calculations [1, 17–23] have been successful in clarifying some aspects of the catalytic properties of the H-cluster. As in previous computational studies [1, 18], CH_3–S is substituted for cysteine, and a H^+ replaces the proximal cubane. Furthermore, computational and experimental research [1, 14, 24–52] on Fe–Fe-hydrogenase H-cluster and synthetic H-cluster-like compounds sheds light on the potential redox states of the Fe–Fe-hydrogenase H-cluster subunit, Fe_p–Fe_d, Fe_p–Fe_d is the reduced hydrogenase H-cluster.

**Scheme 1.** The H-cluster with its proximal cubane and 2Fe subcluster. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
subunit, $\text{Fe}^{II}_p-\text{Fe}^{I}_d$ is the partially oxidized enzyme subunit, and $\text{Fe}^{II}_p-\text{Fe}^{II}_d$ is the fully oxidized, inactive enzyme H-cluster subunit.

The oxidized H-cluster, $\text{Fe}^{II}_p-\text{Fe}^{II}_d$, has OOH$^-$, H$_2$O molecule, or OH$^-$ bound to the Fe$_d^{II}$ site [18, 24]. In our previous investigation [31], we have inferred that a vacant $\text{Fe}^{III}_p-\text{Fe}^{I}_d$ could also be a viable intermediate in H$_2$ synthesis. Regardless of the redox states in [Fe—Fe]-hydrogenase H-cluster, the proximal cubane always retains a 2+ oxidation state, $[\text{Fe}_4S_4]^{2+}$. The partially oxidized H-cluster (containing $\text{Fe}^{III}_p-\text{Fe}^{I}_d$), $H_{ox}$, is the active species of the hydrogenase enzyme having the tendency for protonation (Liu and Hu [18]).

The crystal structures of hydrogenase obtained from Cl. pasteurianum (CP) [15] and Desulfovibrio desulfuricans (Dd) [16] led to a more detailed understanding of the biochemical role of these enzymes. The crystal structure of CPI hydrogenase shows an oxygen species (i.e., either OH$^-$ or H$_2$O) bound to Fe$_d$. However, according to the X-ray crystal structure of CPI, and based on computational results of Tye et al. [24], the enzyme has OOH$^-$ bound to Fe$_d$ in its inactive form. Hence, we endeavor to ascertain if oxygen binding to distal iron (Fe$_d$—O$_2$) can be hindered by residue mutations within the surrounding apoprotein of the catalytic site.

The current investigation comprised two subdivisions: (1) The thermodynamic analysis of the reactions of O$_2$ binding to wild-type and mutated [Fe—Fe]-hydrogenase for the three different oxidation states of the di-iron subcluster: $\text{Fe}^{I}_p-\text{Fe}^{I}_d$, $\text{Fe}^{II}_p-\text{Fe}^{I}_d$, and $\text{Fe}^{II}_p-\text{Fe}^{II}_d$. (2) Geometrical analyses of interatomic distances in Fe$_d$—O$_2$, the extrinsic ligand, O—O, and of CO$_b$ carbon distances to the di-iron atoms. In the Supporting Information, the NBO charges and an electronic analysis discussing the frontier molecular orbitals are presented.

**Methods**

The QM/MM (DFT/UFF) [53] ONIOM [54] method (DFT for the QM region, and the universal force field, UFF, for the MM region, implemented in Gaussian03 [55]) has been used to calculate the reaction thermodynamics, i.e., $\Delta G$, for the reactions of oxygen binding to [Fe—Fe]-hydrogenase. The electronic structure of H-cluster (except the proximal cubane) was described by QM using the DFT Hamiltonian (B3LYP functional [56, 57]), with 6-31+G(d,p) basis set. In accordance with experimental and in silico data, low spin states (singlet, and doublet) and low oxidation states (I, and II) have been selected for the Fe atoms [1, 23].

The Gromacs program [58, 59] was used to add hydrogen atoms, water, and counter ions to the crystal structure of DdH [Brookhaven Protein Data Bank (id.1HFE)]. A 1 nm layer of water has been added to the DdH structure. Sodium cations have been randomly inserted into the solvent to neutralize the negative charges encountered in DdH, e.g., the $-2$ a.u. found on the cubane/cysteine moieties, or in the H-cluster (when needed) [60]. For both basic and acidic amino acids, charges were assigned by the Gromacs program pdb2gmx to be at pH 7. The low layer consists of all metalloenzyme residues as well as its constituent cubanes, i.e., proximal, medial, and distal. The high layer comprised 2Fe subunit, which is a moiety of the H-cluster), and C$_p$ and S$_p$ (of the bridging Cys$^{382}$). Two linking hydrogen atoms were added between C$_p$ and C$_p$ of Cys$^{382}$, and between S$_p$ and a Fe atom of the proximal cubane. The charge equilibration method of the UFF was used to describe the electrostatic interactions within the low layer of the system [61]. The DdH partial charges were obtained using the charge equilibration method (QEeq), whereas the solvent charges were acquired from literature [61] ($q_{O} = -0.706$ a.u. and $q_{H} = 0.353$ a.u.).

ONIOM geometry optimizations have been performed on DdH, with the low layer (MM region) being frozen$^2$, with the exception of the proximal cubane; for the high layer (QM), only the iron atoms, Fe$_p$—Fe$_d$, and the N (of the DTMA bridge) have been kept frozen$^3$.

Residue mutations were carried out within the adjacent apoenzyme environment to the H-cluster in order to hinder O$_2$ from binding to the open coordination site (Fe$_d$) of DdH H-cluster. Residue mutations are composed of deletions and substitutions, which are performed 8 Å radially outward from Fe$_d$. To screen the 30 polar residues located in the 8 Å apoenzyme layer, individual residue deletions were carried out followed by calculations to ascertain what residue substitutions should be.

$^2$Where “frozen” means that x, y, z atom coordinates are kept fixed; “freezing atoms” is practiced to reduce the computational time.

$^3$For the fully and partially oxidized vacant di-iron subunits, additional optimizations have been carried out by freezing these atoms: Fe$_p$—CO$_2$ (where CO$_2$ stands for terminal carbonyl; CO$_2$ is bound to Fe$_p$). The extra optimizations have been done because the above-mentioned di-iron subunits are more likely to undergo CO$_2$ migration.
made to impede O₂ binding to Fe₄. The deletion of a residue was performed by removing the point charges of its atoms from the Gaussian input file. Residue deletions and substitutions were performed for all three di-iron subcluster oxidation states of [Fe–Fe]-hydrogenase H-cluster: Fe₄II–Fe₄II, Fe₄II–Fe₄I, and Fe₄I–Fe₄I.

Results and Discussion

THERMODYNAMICS OF O₂ BINDING TO [Fe–Fe]-HYDROGENASE H-CLUSTER IN GAS PHASE AND PROTEIN ENVIRONMENT

Calculations in Gas Phase and in Wild-Type Enzyme Environment

The results of the QM/MM calculations for the reactions of O₂ binding to [Fe–Fe]-hydrogenase are shown in Table I. Two values of the Gibbs energy are given for wild-type DdH. In the first row are Gibbs energies (ΔG°QM/MM) obtained by taking into account neighboring charges of the 2Fe subunit: i.e., point charges from the proximal cubane ⁴, the MM region of Cys⁵², C of the peptide bond in Gly³⁸¹, and N of Val³⁸³. The values (ΔG°QM/MM) in the second row of Table I were obtained without including the neighboring charges of the 2Fe subunit in the calculation. Sometimes the deletion of MM charges in the vicinity of the QM system is necessary to avoid distortions (artificial polarization) in the wave function induced by these charges. The difference in the wave function polarization (i.e., with or without neighboring charges) is quantified by difference in the natural bond orbital charges [NBO, Figs. 1(a) and (b)]. The strongest effect of the neighboring MM charges is on the NBO charges of S₃ of Cys³⁸² and the linking atom (H₄) attached to it. A detailed analysis of these charges is given in the Supporting Information.

Figure 2 shows O₂ inhibition pathways for all three oxidation states of the H-clusters: Fe₄II–Fe₄II (1), Fe₄II–Fe₄I (3), and Fe₄I–Fe₄I (5), where (1), (3), and (5), are the cluster identifiers. In gas phase, the 1st reaction, 1 → 2, (O₂ binds to the fully oxidized H-cluster (1)) is endergonic (ΔG°gas = +9.8 kcal/mol; gas = gas phase ⁶). Hybrid QM/MM calculations, on the other hand, show that O₂ binding in protein environment occurs exergonically (ΔG°QM/MM = −16.6 kcal/mol; ΔG°QM/MM = −10.6 kcal/mol), confirming the affinity of hydrogenases for O₂ [62].

In gas phase, the 2nd reaction, 3 → 4, starts with the partially oxidized H-cluster (3), Fe₄II–Fe₄I, and the bonding of O₂ to Fe₄I occurs rather exergonic (ΔG°gas = −36.1 kcal/mol). ONIOM results show that in protein environment O₂ binds spontaneously to the H-cluster when neighboring charges are included (Fe₄II–Fe₄I, ΔG°QM/MM = −7.9 kcal/mol), but the binding reaction is nonspontaneous

---

Table I

<table>
<thead>
<tr>
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<th>O₂ +</th>
<th>O₂ +</th>
<th>O₂ +</th>
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<tr>
<td>ΔGln³⁸⁸</td>
<td>−10.8</td>
<td>+2.5</td>
<td>−20.4</td>
</tr>
</tbody>
</table>

⁴ Except for the cubane sulfur (S₃,d) situated diagonally from the cubane Fe (bound to cysteinyl sulfur of Cys³⁸²).

⁵ The gas phase results are reproduced from Ref. [17].
(\(\Delta G_{\text{QM/MM}} = +2.6 \ \text{kcal/mol}\)) when the neighboring charges are not included.

The 3rd reaction in gas phase, \(5 \rightarrow 6\), starts with the reduced H-cluster (5), \(\text{Fe}_\text{p}^{1}\text{--Fe}_\text{d}^{1}\), the reaction occurs spontaneously (\(\Delta G_{\text{gas}} = -36.0 \ \text{kcal/mol}\)) and has almost identical Gibbs energy as reaction 3 \(\rightarrow 4\), probably because both loci of oxygen binding (\(\text{Fe}_\text{d}^{1}--\text{O}_2\)) are on similar oxidized species, \(\text{Fe}_\text{d}^{1}\). However, the QM/MM calculations for \(5 \rightarrow 6\) show a difference between the enzyme (\(\Delta G_{\text{QM/MM}} = -20.7 \ \text{kcal/mol}\); \(\Delta G_{\text{QM/MM}} = -20.5 \ \text{kcal/mol}\)) and gas phase results (\(\Delta G_{\text{gas}} = -36.0 \ \text{kcal/mol}\)) Gibbs energies.

In Figure 2, the gas phase protonation reaction \(5 \rightarrow 7\) is very exergonic (\(\Delta G_{\text{gas}} = -220.6 \ \text{kcal/mol}\)), essentially because the charge on H-cluster 5 is \(-2\) a.u.

ONIOM calculations also show a very high H\(^+\) affinity (\(\Delta G_{\text{QM/MM}} = -219.2 \ \text{kcal/mol}\)) for the hydrogenase H-cluster, which is close to the gas phase result (\(\Delta G_{\text{gas}} = -220.6 \ \text{kcal/mol}\)). The thermodynamic results in Figure 2 demonstrate that most reactions considered for the hydrogenase H-cluster proceed exergonically with the exception of \(1 \rightarrow 2\) in gas phase. Thus, the calculations on the wilde-type [Fe—Fe] hydrogenase are in agreement with experimental observations and confirm the inhibition of [Fe—Fe]-hydrogenase by molecular oxygen.

**Calculations in Mutated Enzyme Environment**

The thermodynamic calculations of O\(_2\) binding to the H-cluster in gas phase and protein environ-
ment suggest that the enzyme electric field modulates the reactivity of the H-cluster toward $O_2$, and thus is responsible for the inhibition of [Fe$–$Fe$]$-hydrogenase by molecular oxygen. Thus, thoughtful modification of the enzyme electric field by residue mutation may advert or weaken the $O_2$ binding to the H-cluster. This idea is the basis of the study presented here.

Residue screening in a protein layer (8 Å) surrounding the H-cluster has been carried out to gauge the effect of point mutations on the strength of $O_2$ binding to H-cluster. First, we performed residue deletions to gauge the effect of the electric field of the residues targeted for substitution on the reaction of $O_2$ binding, and then we performed residue substitutions for residues that showed significant effect upon deletion.

For $O_2$ binding to Fe$^\text{I}_{\text{d}}$ (of the oxidized biferrous hydrogenase H-cluster subsite, Fe$_p^\text{II}$–Fe$_d^\text{II}$, (1)), the results obtained are a function of stereoelectronic effects from the juxtaposed residues on the catalytic site. Both neutral polar and charged residue deletions provided good results, e.g., $\Delta$Ser$^{62,6}$, $\Delta$Asp$^{144}$, $\Delta$Glu$^{146}$, $\Delta$Asp$^{150}$, $\Delta$Thr$^{152}$, and $\Delta$Ser$^{202}$ gave $\Delta G_{\text{QM/MM}} = -9.0$ kcal/mol, $\Delta G_{\text{QM/MM}} = -8.4$ kcal/mol, $\Delta G_{\text{QM/MM}} = -8.7$ kcal/mol, $\Delta G_{\text{QM/MM}} = -9.4$ kcal/mol, $\Delta G_{\text{QM/MM}} = -9.2$ kcal/mol, and $\Delta G_{\text{QM/MM}} = -7.9$ kcal/mol, respectively.

$O_2$ is hindered from binding to Fe$_d^\text{I}$ of the partially oxidized di-iron subsite (Fe$_p^\text{II}$–Fe$_d^\text{I}$). Endergonic binding reactions have been identified for all tried residue deletions (Table I), except for the following: $\Delta$Thr$^{152}$, $\Delta$Ly$^{257}$, $\Delta$Thr$^{257}$, $\Delta$Thr$^{259}$, $\Delta$Ser$^{289}$, and $\Delta$Thr$^{299}$, which gave $\Delta G_{\text{QM/MM}} = -3.2$ kcal/mol, $\Delta G_{\text{QM/MM}} = -2.7$ kcal/mol, $\Delta G_{\text{QM/MM}} = -2.9$ kcal/mol, $\Delta G_{\text{QM/MM}} = -3.0$ kcal/mol, $\Delta G_{\text{QM/MM}} = -2.9$ kcal/mol, and $\Delta G_{\text{QM/MM}} = -2.8$ kcal/mol, respectively.

An improved trend toward impeding $O_2$ binding to Fe$_d^\text{I}$, of the fully reduced di-iron subsite (Fe$_p^\text{I}$–Fe$_d^\text{I}$), has been observed for residue deletions (Table I): $\Delta$Thr$^{152}$ and $\Delta$Ser$^{202}$, which gave $\Delta G_{\text{QM/MM}} = -18.6$ kcal/mol, and $\Delta G_{\text{QM/MM}} = -17.3$ kcal/mol, respectively. Table I shows that the deletion of charged amino acid residues like Asp$^{144}$ and Glu$^{274}$ has a similar effect ($\Delta G_{\text{QM/MM}} = +4.6$ and $+4.7$ kcal/mol, respectively) on $O_2$ binding to Fe$_p^\text{I}$–Fe$_d^\text{I}$, as the deletion of polar but neutral amino acid residues like Thr$^{152}$ and Ser$^{202}$ ($\Delta G_{\text{QM/MM}} = +4.1$ and $+3.9$ kcal/mol, respectively), which seems rather intriguing. A closer look at the distribution of the amino acid residues targeted for deletion in the 8 Å layer around the di-iron catalytic unit reveals that the charged residues Asp$^{144}$ and Glu$^{274}$ are about 10 Å away from distal Fe, while Thr$^{152}$ and Ser$^{202}$ are about 3 Å closer. This difference in location with respect to the H-cluster explains why the deletion of two charged residues, further away, has a similar effect on $O_2$ binding as the deletion of two polar residues, which are much closer to H-cluster. The residues interlaced between Asp$^{144}$/Glu$^{274}$ and the catalytic site screen the bare charge of these charged residues, thus diminishing its effect on the electronic structure of the di-iron unit. Because charged residues have a stronger effect on their local environment than polar residues, it is expected that polar/non-polar residues are better targets for mutations because their replacement should be less detrimental to the overall folding of the enzyme. Thus, we selected neutral polar residues for mutations.

With the positive results obtained from residue deletions, we were now able to carry out residue substitutions (Table II). Two residue deletions ($\Delta$Thr$^{152}$ and $\Delta$Ser$^{202}$), which showed significant effect on the Gibbs energy of $O_2$ binding for all oxidation states of the di-iron H-cluster subunit, were followed by mutations to alanine, i.e., Thr$^{152}$Ala and Ser$^{202}$Ala. The dual residue deletions, $\Delta$Thr$^{152}$ and $\Delta$Ser$^{202}$, impede further $O_2$ binding to the H-cluster subunit Fe$_p^\text{II}$–Fe$_d^\text{I}$ ($\Delta G_{\text{QM/MM}} = +5.4$ kcal/mol). However, for the oxidation states Fe$_p^\text{II}$–Fe$_d^\text{II}$ and Fe$_p^\text{I}$–Fe$_d^\text{I}$, only a slight enhancement in $O_2$ inhibition has been observed (+2.2 and $+4.4$ kcal/mol, respectively). The simultaneous mutations to alanine (Thr$^{152}$Ala and Ser$^{202}$Ala) give better $O_2$ inhibition results ($\Delta G_{\text{QM/MM}} = -9.2$ kcal/mol for Fe$_p^\text{II}$–Fe$_d^\text{II}$, $\Delta G_{\text{QM/MM}} = +4.2$ kcal/mol for Fe$_p^\text{II}$–Fe$_d^\text{I}$, and $\Delta G_{\text{QM/MM}} = -18.1$ kcal/mol for Fe$_p^\text{I}$–Fe$_d^\text{I}$).

### Table II

Gibbs energies (kcal/mol) for $O_2$ binding to wild-type DdH and to DdH modified by residue deletion.

<table>
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<th>Fell–Fell $+ O_2$</th>
<th>Fell–Fell $+ O_2$</th>
<th>Fell–Fell $+ O_2$</th>
</tr>
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<td>-20.5</td>
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<tr>
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<td>-8.4</td>
<td>+5.4</td>
<td>-16.1</td>
</tr>
<tr>
<td>$\Delta$Thr$^{152}$, $\Delta$Ser$^{202}$ (at 100°C)</td>
<td>-5.6</td>
<td>+7.9</td>
<td>-12.9</td>
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<tr>
<td>Thr$^{152}$Ala, Ser$^{202}$Ala</td>
<td>-9.2</td>
<td>+4.2</td>
<td>-18.1</td>
</tr>
</tbody>
</table>

$s = $ DdH small chain.
TABLE III
Geometrical results for wild-type DdH H-cluster.
Interatomic distances (Å) between Fe₆ and CO₆, Fe₆ and CO₆, Fe₆ and O₆, and O₆−O₆, and the angle Fe₆−O₆−O₆ before and after O₂ binding.

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<tr>
<th></th>
<th>Fe²⁺Fe²⁺</th>
<th>Fe²⁺Fe₁</th>
<th>Fe¹Fe¹</th>
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<td>Before O₂</td>
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<td>1.807</td>
<td>1.924</td>
<td>1.935</td>
</tr>
<tr>
<td>Fe₆−CO₆</td>
<td>2.287</td>
<td>1.924</td>
<td>1.945</td>
</tr>
<tr>
<td>O₁−Fe₆</td>
<td>1.729</td>
<td>1.840</td>
<td>1.808</td>
</tr>
<tr>
<td>O₁−O₁</td>
<td>1.276</td>
<td>1.281</td>
<td>1.373</td>
</tr>
<tr>
<td>Fe₆−O₁−O₁</td>
<td>137.0</td>
<td>160.6</td>
<td>126.0</td>
</tr>
</tbody>
</table>

Additionally, it is known that certain organisms containing [Fe–Fe]-hydrogenases thrive around sub-oceanic thermal vents [3]. Hence, at a temperature of 100°C intercalated with hydrogenase mutations (Table II), QM/MM results indicate that the extrinsic O₂ binding to metalloenzyme is further reduced ($\Delta G_{Q/MM} = −5.6$ kcal/mol for Fe₆−Fe₆, $\Delta G_{Q/MM} = +7.9$ kcal/mol for Fe₆−Fe₆, and $\Delta G_{Q/MM} = −12.9$ kcal/mol for Fe₆−Fe₆).

DdH GEOMETRICAL READJUSTMENT UPON OXIDATION

In this section, the Gibbs energies for the reaction of O₂ binding to wild-type DdH are correlated with geometrical parameters such as interatomic distances and bond angles (Table III). The iron-carbon distances in the Fe₆−CO₆ subunit are investigated for all three oxidation states of the di-iron subunits.

The iron-carbon distance in Fe₆−CO₆ (Fe₆−Fe₆) becomes smaller, 1.925 Å (1) → 1.807 Å (2), upon O₂ binding concomitant with Fe₆−CO₆ bond elongation, 1.942 Å (1) → 2.287 Å (2), which generally indicates an increased bonding strength for an exogenous ligand [31] (i.e., O₂). For Fe₆−Fe₆, the bond distance Fe₆−CO₆ becomes smaller, 1.939 Å (3) → 1.924 Å (4), upon O₂ binding while a bond elongation is observed for Fe₆−CO₆, 1.908 Å (3) → 1.924 Å (4). For the reduced di-iron subcluster (Fe₆−Fe₆) the bond distance Fe₆−CO₆ becomes smaller, 1.942 Å (5) → 1.935 Å (6), upon O₂ binding, while Fe₆−CO₆ increases, 1.826 Å (5) → 1.945Å (6). The above geometrical analysis concludes that for all oxidation states of Fe₆−Fe₆ the bond between the carbon of the bridging carbonyl (CO₆) and Fe₆ becomes longer and the bond between CO₆ and Fe₆ becomes shorter, upon O₂ binding to the catalytic site.

Next, an analysis is presented for the interatomic distances between distal iron and oxygen, and between oxygen atoms, relative to Gibbs energy of O₂ binding to all three di-iron oxidation states.

For the Fe₆−Fe₆ subcluster, the iron-oxygen distance, Fe₆−O₆, is rather small (1.729 Å; Table III), which suggests a strong bonding ($\Delta G_{Q/MM} = −10.6$ kcal/mol; 1 → 2) between the distal iron and the oxygen atom bound to it. The interoxygen (O₁−O₁) bond distance is 1.276 Å, which corresponds to a bond order between a single and double bond.

In the case of the active di-iron subcluster, Fe₆−Fe₆, the Fe₆−O₆ bond distance is ca. 6% longer (1.840 Å) than Fe₆−O₆ interatomic distance in Fe₆−Fe₆−O₆, giving rise to a weaker bond ($\Delta G_{Q/MM} = +2.6$ kcal/mol; 3 → 4) between the distal iron and oxygen. The O₁−O₁ bond distance is 1.281 Å, which is relatively close to the O₁−O₁ bond for Fe₆−Fe₆−Fe₆ subcluster.

In Fe₆−Fe₆, the O₁−O₁ bond distance is relatively larger, 1.373 Å, which suggests that π-backdonation occurs between a filled d-orbital of Fe₆ and the empty π-orbital of O₆. Out of the three di-iron oxidation states, only the reduced di-iron subcluster (Fe₆−Fe₆) has attributes of π-backdonation, i.e., the O₁−O₁ bond order is intermediate between a double and a single bond order, and the O₁−O₁ bond is elongated (1.373 Å).

Finally, the Fe₆−O₁−O₁ angle varies as the oxidation states decrease: Fe₆−Fe₆−O₆−O₆ (137.0°), Fe₆−Fe₆−O₆−O₆ (160.5°), and Fe₆−Fe₆−O₁−O₁ (126.0°) in conjunction with effects of the nearby electric field of the apoprotein.

7The π-backonation agrees with the Gibbs energy results form Table I. For example, the Fe₆−Fe₆ subcluster has an exergonic Gibbs energy ($\Delta G_{Q/MM} = −16.6$ kcal/mol), which can be improved however by DdH mutations such as residue deletions and substitutions, since there is only slight π-backdonation present. The bond Fe₆−O₁ is still relatively weak ($\Delta G = −7.9$ kcal/mol) for Fe₆−Fe₆ subcluster. However, for the reduced Fe₆−Fe₆ subcluster, the π-backonation makes the oxygen bond very strong, thus making its elimination rather difficult (even by means of DdH mutations, Table II).
Conclusion

The QM calculations on the gas phase H-cluster in different oxidation states of Fe$_p$—Fe$_d$ subunit show that O$_2$ binding to the fully oxidized H-cluster (Fe$_p^{II}$—Fe$_d^{II}$) is exergonic, and endergonic for the partially oxidized (Fe$_p^{II}$—Fe$_d^{I}$) and reduced (Fe$_p^{I}$—Fe$_d^{I}$) H-clusters.

On the other hand, the QM/MM calculations on the wild-type [Fe—Fe]-hydrogenase confirm that the resting state of the enzyme (Fe$_p^{II}$—Fe$_d^{II}$) is inhibited by O$_2$. In addition, the O$_2$ binding to the partially oxidized (Fe$_p^{II}$—Fe$_d^{I}$) hydrogenase is slightly endergonic, but is exergonic for the reduced oxidation state (Fe$_p^{I}$—Fe$_d^{I}$). The contrast between the calculation results in gas phase and protein environment suggest a dramatic effect of the enzyme electric field on the O$_2$ binding reaction. Thus, we explored the modulation of this electric field by performing point mutations in an 8 Å protein layer surrounding the H-cluster. First, residue deletions have been performed, one by one. Then, from clues obtained from these residue deletions, residue substitutions have been carried out. For Fe$_p^{II}$—Fe$_d^{II}$, both the neutral polar residue and the charged residue deletions (ΔSer$^{62s}$, ΔAsp$^{144}$, ΔGlu$^{146}$, ΔAsp$^{150}$, ΔThr$^{152}$, and ΔSer$^{202}$) led to a decreased binding of O$_2$. For Fe$_p^{II}$—Fe$_d^{I}$ residue deletions ΔGlu$^{374}$, ΔAsp$^{144}$, ΔSer$^{177}$, and ΔThr$^{152}$ and for Fe$_p^{I}$—Fe$_d^{I}$ residue deletions ΔThr$^{152}$ and ΔSer$^{202}$ hinder O$_2$ binding to Fe$_d^{I}$.

As expected, residue substitutions Thr$^{152}$Ala and Ser$^{202}$Ala affect O$_2$ binding to the same extent as the corresponding deletions (ΔThr$^{152}$ and ΔSer$^{202}$).

Finally, the fact that one substitutes nonpolar amino acid residues (Thr$^{152}$Ala and Ser$^{202}$Ala) for some polar ones juxtaposed to the catalytic site (the H-cluster) seems to be sufficient cause to hinder the binding of O$_2$ to hydrogenase H-cluster. This shows that well thought modulation of the enzyme electric field accomplished by point mutations can be used to advert hydrogenase inactivation by molecular oxygen. Hence, DdH mutations open up new research opportunities along these lines.

ACKNOWLEDGMENTS

Computational resources have been provided by the National Center for Supercomputer Applications (University of Illinois) and the Ohio Supercomputer Center.

References