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Reactivation Pathway of the Hydrogenase H-Cluster: Density Functional Theory Study

STEFAN MOTIU, DANIELA DOGARU, VALENTIN GOGONEA

Introduction

Hydrogenases are a family of enzymes that reversibly catalyze the transformation of protons and electrons to molecular hydrogen \((2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2)\). The exploration for alternative energy sources has engendered great interest in hydrogenase research [1–6]. However, these enzymes appear to be inactivated by exogenous ligands [7], such as \(\text{O}_2\) and \(\text{OH}^-\). Water also binds to the active site, in the resting state, of the enzyme [8, 9], \(\text{Fe}^2_\text{p}^\text{II} - \text{Fe}^2_\text{d}^\text{II}\) (where \(\text{Fe}_\text{p}\) is the proximal iron, and \(\text{Fe}_\text{d}\) is the distal iron).

The recently detailed structures of Fe-only hydrogenases, from *Clostridium pasteurianum* (CPI) [10,11] and *Desulfovibrio desulfuricans* (DdH) [12,13],
offer new opportunities for understanding their functions via biochemical pathways [7, 14]. It is now possible to determine the inhibitory mechanisms of O₂ and OH⁻ by performing density functional theory (DFT) calculations on the active site of these enzymes, i.e., the H-cluster. This cluster is composed of two iron atoms bridged by the di(thiomethyl)amine (DTMA) group, coordinated by endogenous cyanide, carbon monoxide, and the bridging carbonyl (CO_b) ligands. At the proximal metal, a cysteine-S bridging occurs to a [4Fe–4S] cubane, but in our investigation cysteine is replaced with CH₃–S, and the cubane is exchanged with H⁺ (Fig. 1).

By performing DFT calculations on the H-cluster, with OH⁻ bound to Fe₃ (redox states, Feᴵ⁻Feᴵ, Feᴵ⁻Feᴵᴵ, Feᴵᴵ⁻Feᴵᴵ), Liu and Hu [7] have inferred, based on agreement between the calculated and experimental vibrational frequencies of the three endogenous CO ligands, that OH⁻ is the oxygen species that inhibits hydrogenases.

**Methods**

The electronic structure of the H-cluster (Fe-only, and Ru-modified) was investigated by quantum mechanics (Gaussian 03 [15]), using the DFT method (B3LYP functional [16, 17]), with a variety of bases sets. Exploratory calculations have been performed with the 6-311G(d,p) basis set, and further refined with 6-311G(d,p) basis set. For Fe and Ru, an effective core potential (ECP) with a double-zeta polarization basis set (LANL2DZ) [18, 19] was used. In accordance with experimental and in-silico data, we selected low spin states (singlet and doublet) and low oxidation states (I and II) for the metal atoms [20–25].

**Results and Discussion**

Liu and Hu [7] showed (reactivation pathway I; Scheme 1) that Feᴵᴵ⁻OH⁻ (1) can be further reduced to Feᴵ⁻OH⁻ (2) but we found that the electron transfer is endothermic (ΔH = +12.31 kcal/mol; Table I) because the H-cluster (1) is already negatively charged (−1 a.u.). Feᴵ⁻OH⁻ (2) can be easily protonated to Feᴵ⁻OH₂ (3) because its proton affin-

**TABLE I**

<table>
<thead>
<tr>
<th>Metal combination</th>
<th>Reaction enthalpy</th>
<th>Reaction enthalpy</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 → 2</td>
<td>2 → 3</td>
<td>3 → 4</td>
</tr>
<tr>
<td>Fe–Fe</td>
<td>+12.31b</td>
<td>−410.80</td>
<td>+5.92</td>
</tr>
<tr>
<td></td>
<td>+12.75c</td>
<td>−411.64</td>
<td>+5.89</td>
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<td>Fe–Ru</td>
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<td>−406.49</td>
<td>+3.42</td>
</tr>
<tr>
<td></td>
<td>+10.37</td>
<td>−406.92</td>
<td>+3.38</td>
</tr>
<tr>
<td>Ru–Fe</td>
<td>+14.14</td>
<td>−413.96</td>
<td>+4.93</td>
</tr>
<tr>
<td></td>
<td>+14.51</td>
<td>−414.67</td>
<td>+4.89</td>
</tr>
<tr>
<td>Ru–Ru</td>
<td>+15.90</td>
<td>−412.58</td>
<td>+11.93</td>
</tr>
<tr>
<td></td>
<td>+16.12</td>
<td>−412.93</td>
<td>+11.80</td>
</tr>
</tbody>
</table>

*In kcal/mol.

b Results obtained at B3LYP/6-31+G(d,p) level.

c Results obtained at B3LYP/6-311+G(d,p) level.

The process whereby the H-cluster is set free, once again, of exogenous ligands, e.g., OH⁻, H₂O, at the active site, Fe_d⁻, in its reduced form. Thus, in its reactivated state, the H-cluster is enabled to engage in its (former) catalytic H₂ redox activity.

Calculations on Fe(I) have been previously reported in Refs. [7, 14, 21–24].
ity is +410.8 kcal/mol. The water dissociation from Fe²⁺–OH₂ (3) is also endothermic (ΔH = +5.92 kcal/mol).

Scheme 2 shows the reactivation pathway II in which the protonation step occurs first. This step proceeds because the proton affinity of Fe²⁺–OH⁻ (1) is still very large (+326.95 kcal/mol, Table II). Furthermore, Scheme 2 shows that water dissociation from Fe²⁺–OH₂ (2') is also exothermic (ΔH = −3.22 kcal/mol). In contrast, Cao and Hall [22] found that the removal of water from the distal iron of Fe²⁺–Fe²⁺ is rather endothermic (ΔH = +23 kcal/mol).

The difference in the reaction enthalpy calculated by Cao and Hall [22], relative to our result, may stem from the fact that the optimized structure, Fe²⁺–Fe²⁺ (3'), in Cao and Hall’s study, has the bridging carbonyl ligand (CO₇) midway between Fe₇ and Fe₆, which makes the H-cluster higher in energy than it is when CO₇ is bound (asymmetrically) closer to Fe₇ [Scheme 2, (3')]. However, we were unable to find a stationary point (energy minimum) at the B3LYP/6-31+G(d,p) level for the structure with symmetrically bound CO₇, but we obtained a partially optimized structure by constraining only the distance between CO₇ and Fe₆—the breaking bond—at 2.147 Å (the distance between CO₇ and Fe₆ was reduced from 2.040 Å to 1.873 Å during optimization). This quasi-symmetrical structure is 14 kcal mol⁻¹ higher in energy than the structure with CO₇ bound asymmetrically (3') to the two irons. Thus, a symmetrically CO₇-bound structure is expected to be even higher in energy. Hence, the removal of H₂O (Scheme 2) is exothermic, as the CO₇ has been shifted toward Fe₆, and this facilitates exogenous ligand bond breaking [14]. Another structural detail contributing to the difference in the reaction enthalpies, 2' → 3', is that in the reactivation pathway of Scheme 2 (same for Liu and Hu [7]), the nonbridging sulfur bound to Fe²⁺ is protonated. Also, owing to the different levels of theory used by each investigating group, inevitably different optimized geometries are obtained.

The result of this investigation, regarding water removal from Fe²⁺, corroborates an antithetical reactivation pathway, that is, the reduction of Fe²⁺–Fe²⁺ (3') is exothermic (unlike Scheme 1 [22]), for the CO₇ is bound only to Fe²⁺, and not to Fe²⁺. Our results indicate that water removal from Fe²⁺ is facilitated by concerted bond breaking of CO₇ from Fe²⁺ and bond contraction between CO₇ and Fe²⁺ (as the bridging carbonyl migrates toward Fe²⁺) (Table III). The reactivation of the H-cluster is complete upon reduction [26] of Fe²⁺ to Fe⁰. This reduction process is highly exothermic (ΔH = −62.41 kcal/mol; Table II).

This reaction pathway (Scheme 2) thermodynamically favors the reactivation of the H-cluster, and suggests that the H-cluster may not be permanently inhibited by OH⁻, or H₂O, nonetheless. It appears that as long as the H-cluster is supplied protons, its reactivation shall continue.

The following bimetal combinations within the H-cluster, i.e., Fe₇–Fe₇, Fe₇–Ru₇, Ru₇–Fe₇, and Ru₇–Ru₇, were theoretically investigated (Tables I and II) to elucidate which combinations are less sensitive to OH⁻ inhibition, and to determine whether the varied metal combinations perform thermodynamically better than the Fe–Fe H-cluster.

<table>
<thead>
<tr>
<th>Metal combination</th>
<th>Reaction enthalpya</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 → 2'</td>
</tr>
<tr>
<td>Fe–Fe</td>
<td>−326.95b</td>
</tr>
<tr>
<td></td>
<td>−327.55c</td>
</tr>
<tr>
<td>Fe–Ru</td>
<td>−331.14</td>
</tr>
<tr>
<td></td>
<td>−331.50</td>
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<tr>
<td>Ru–Fe</td>
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<td></td>
<td>−330.21</td>
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<tr>
<td>Ru–Ru</td>
<td>−328.13</td>
</tr>
<tr>
<td></td>
<td>−328.52</td>
</tr>
</tbody>
</table>

a In kcal/mol.

b Results obtained at B3LYP/6-31+G(d,p) level.

c Results obtained at B3LYP/6-311+G(d,p) level.
Table I lists reaction enthalpies for the reactivation pathway I. For Fe₆–Ru₂ modified H-cluster the electron transfer is slightly less endothermic (-2.29 kcal/mol) than for Fe₆–Fe₄ H-cluster. The proton affinities for Ru₆–Fe₄, and Ru₆–Ru₂ are slightly larger than for Fe-only, except Fe₆–Ru₂. However, in the H₂O removal step, the bimetals Fe₆–Ru₂ and Ru₆–Fe₄ release water more readily than the Fe-only cluster. Conversely, calculations on Ru-only H-cluster indicate that Ru₄ binds water more firmly than Fe₄ (Table I).

In contrast, for the reactivation pathway II, protonation of bimetal combinations, i.e., Fe₆–Ru₂, Ru₆–Fe₄, and Ru-only, is highly exothermic (similar to the Fe-only H-cluster). However, water removal is endothermic for these bimetal combinations. Subsequently, the reduction process (Scheme 2) necessitates similar enthalpies of reaction for most bimetal combinations (Table II) mentioned above except for Ru₆–Fe₄ which is slightly more exothermic.

Conclusions

Reactivation pathway I consists of an endothermic electron transfer step, followed by an exothermic protonation step, and then an endothermic water removal step. For reactivation pathway II, the H-cluster protonation occurs first, followed by water removal, and then by electron transfer with all steps being exothermic.

Finally, we propose a reaction pathway for the reactivation of the hydrogenase H-cluster, in which all individual reaction steps are thermodynamically favored (Scheme 2).

Acknowledgments

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References
