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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 O_2 , and OH^- . Water also binds to the active site, in the resting state, of the enzyme [8, 9], $\text{Fe}_\text{p}^\text{II}-\text{Fe}_\text{d}^\text{II}$ (where Fe_p is the proximal iron, and Fe_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],

FIGURE 1. H-cluster structure (M = Fe, Ru).

offer new opportunities for understanding their functions via biochemical pathways [7, 14]. It is now possible to determine the inhibitory mechanisms of O_2 , 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(thio-methyl)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 CH_3-S , and the cubane is exchanged with H^+ (Fig. 1).

By performing DFT calculations on the H-cluster, with OH^- , and O_2 bound to Fe_d (redox states, Fe^I-Fe^I , Fe^I-Fe^{II} , $Fe^{II}-Fe^{II}$), 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.

SCHEME 1. Reactivation pathway I of Fe-only, and Ru-modified H-cluster.

TABLE I
Reaction enthalpies for elementary reaction steps of the reactivation pathway I.

Metal combination	Reaction enthalpy ^a		
	1 → 2	2 → 3	3 → 4
Fe-Fe	+12.31 ^b	-410.80	+5.92
	+12.75 ^c	-411.64	+5.89
Fe-Ru	+10.02	-406.49	+3.42
	+10.37	-406.92	+3.38
Ru-Fe	+14.14	-413.96	+4.93
	+14.51	-414.67	+4.89
Ru-Ru	+15.90	-412.58	+11.93
	+16.12	-412.93	+11.80

^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.

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 6-31+G(*d,p*) basis set, and further refined with 6-311+G(*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 (reactivation¹ pathway I; Scheme 1) that $Fe_d^{II}-OH^-$ (1) can be further reduced to $Fe_d^I-OH^-$ (2),² but we found that the electron transfer is endothermic ($\Delta H = +12.31$ kcal/mol; Table I) because the H-cluster (1) is already negatively charged (-1 a.u.). $Fe_d^I-OH^-$ (2) can be easily protonated to $Fe_d^I-OH_2$ (3) because its proton affin-

¹The process whereby the H-cluster is set free, once again, of exogenous ligands, e.g., OH^- , H_2O , at the active site, Fe_d^I , in its reduced form. Thus, in its reactivated state, the H-cluster is enabled to engage in its (former) catalytic H_2 redox activity.

²Calculations on Fe(I) have been previously reported in Refs. [7, 14, 21–24].

SCHEME 2. Reactivation pathway II of Fe-only, and Ru-modified H-cluster.

ity is +410.8 kcal/mol. The water dissociation from $\text{Fe}_d^{\text{II}}\text{-OH}_2$ (3) is also endothermic ($\Delta H = +5.92$ kcal/mol).

Scheme 2 shows the reactivation pathway II in which the protonation step occurs first. This step proceeds because the proton affinity of $\text{Fe}_d^{\text{II}}\text{-OH}^-$ (1) is still very large (+326.95 kcal/mol, Table II). Furthermore, Scheme 2 shows that water dissociation from $\text{Fe}_d^{\text{II}}\text{-OH}_2$ (2') is also exothermic ($\Delta H = -3.22$ kcal/mol). In contrast, Cao and Hall [22] found that the removal of water from the distal iron of $\text{Fe}_p^{\text{II}}\text{-Fe}_d^{\text{II}}$ is rather endothermic ($\Delta H = +23$ 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, $\text{Fe}_p^{\text{II}}\text{-Fe}_d^{\text{II}}$ (3'), in Cao and Hall's study, has the bridging carbonyl ligand (CO_b) midway between Fe_p and Fe_d , which makes the H-cluster higher in energy than it is when CO_b is bound (asymmetrically) closer to Fe_d [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 CO_b , but we obtained a partially optimized structure by constraining only the distance between CO_b and Fe_p —the breaking bond—at 2.147 Å (the distance between CO_b and Fe_d 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 CO_b bound asymmetrically (3') to the two irons. Thus, a symmetrically CO_b -bound structure is expected to be even higher in energy. Hence, the removal of H_2O (Scheme 2) is exothermic, as the CO_b has been shifted toward Fe_d , and this facilitates exogenous ligand bond breaking

[14]. Another structural detail contributing to the difference in the reaction enthalpies, $2' \rightarrow 3'$, is that in the reactivation pathway of Scheme 2 (same for Liu and Hu [7]), the nonbridging sulfur bound to Fe_p^{II} 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 Fe_d^{II} , corroborates an antithetical reactivation pathway, that is, the reduction of $\text{Fe}_p^{\text{II}}\text{-Fe}_d^{\text{II}}$ (3') is exothermic (unlike Scheme 1 [22]), for the CO_b is bound only to Fe_d^{II} , and not to Fe_p^{II} . Our results indicate that water removal from Fe_d^{II} is facilitated by concerted bond breaking of CO_b from Fe_p^{II} and bond contraction between CO_b and Fe_d^{II} (as the bridging carbonyl migrates toward Fe_d^{II}) (Table III). The reactivation of the H-cluster is complete upon reduction [26] of Fe_d^{II} to Fe_d^{I} . This reduction process is highly exothermic ($\Delta H = -62.41$ 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 H_2O , 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., $\text{Fe}_p\text{-Fe}_d$, $\text{Fe}_p\text{-Ru}_d$, $\text{Ru}_p\text{-Fe}_d$, and $\text{Ru}_p\text{-Ru}_d$, 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 II
Reaction enthalpies for elementary reaction steps of the reactivation pathway II.

Metal combination	Reaction enthalpy ^a		
	1 $\rightarrow$ 2'	2' $\rightarrow$ 3'	3' $\rightarrow$ 4
Fe-Fe	-326.95 ^b	-3.22	-62.41
	-327.55 ^c	-3.19	-62.25
Fe-Ru	-331.14	+5.13	-67.02
	-331.50	+4.96	-66.63
Ru-Fe	-329.56	+10.26	-75.59
	-330.21	+10.46	-75.52
Ru-Ru	-328.13	+6.06	-62.67
	-328.52	+5.89	-62.38

^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 III

Bond lengths^a (M_p -CO_c and M_d -CO_c) for the reactant, product, and intermediates that appear in reactivation pathways I and II of H-cluster of hydrogenase.

Metal combination	Compound					
	1	2	3	4	2'	3'
Fe-Fe	2.341 ^b	2.817	2.013	2.232	2.411	3.067
	1.829 ^c	1.807	1.945	1.850	1.845	1.819
Fe-Ru	2.541	2.935	2.161	3.710	2.661	3.250
	1.935	1.922	1.977	1.902	1.909	1.915
Ru-Fe	2.141	2.083	2.072	2.110	1.942	1.991
	1.961	2.005	1.984	1.947	2.503	2.487
Ru-Ru	2.458	2.245	2.159	2.170	2.609	3.362
	1.966	2.032	2.019	2.009	1.930	1.918

^a In Å.

^b Results obtained at B3LYP/6-31+G(d p) level for M_p -CO_c.

^c Results obtained at B3LYP/6-31+G(d p) level for M_d -CO_c.

Table I lists reaction enthalpies for the reactivation pathway I. For Fe_p - Ru_d modified H-cluster the electron transfer is slightly less endothermic (+2.29 kcal/mol) than for Fe_p - Fe_d H-cluster. The proton affinities for Ru_p - Fe_d , and Ru_p - Ru_d are slightly larger than for Fe-only, except Fe_p - Ru_d . However, in the H₂O removal step, the bimetal Fe_p - Ru_d and Ru_p - Fe_d release water more readily than the Fe-only cluster. Conversely, calculations on Ru-only H-cluster indicate that Ru_d binds water more firmly than Fe_d (Table I).

In contrast, for the reactivation pathway II, protonation of bimetal combinations, i.e., Fe_p - Ru_d , Ru_p - Fe_d , 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 Ru_p - Fe_d 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).

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