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A Unifying Structural and Electronic Concept for Hmd and [FeFe] Hydrogenase Active Sites

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The hydrogenases [FeFe] and Hmd feature at first sight rather different active sites. A closer inspection reveals striking similarities, which allow us to define swapped ligand spheres in such a way that the single active iron center of Hmd functions in a first-shell ligand environment resembling the reacting iron atom in [FeFe] hydrogenase and vice versa. These redesigned ligand environments can be conveniently studied with quantum chemical methods and point to general reactivity principles for iron centers with hydrogenase activity.

Introduction

Hydrogenases mediate the formation or oxidation of molecular hydrogen.[1] Three different types of these enzymes are known: the [NiFe] hydrogenase, the [FeFe] hydrogenase, and the [Fe] hydrogenase, also called dihydrogen-forming methylenetetrahydromethanopterin dehydrogenase (Hmd). The molecular structures of their active sites exhibit a remarkable molecular diversity. The Hmd's active site contains only a single iron center.[2] The experimental structure was a prerequisite for quantum chemical investigations into its reaction mechanism.[3] At this iron center, hydrogen transformation takes place. It is surrounded by ligands of which one is a chelate ligand with a σ-donating pyridine nitrogen atom that is substituted in the ortho position by a very unusual acyl ligand coordinating via its nucleophilic carbon atom. By contrast, the [FeFe] hydrogenase consists of a dinuclear sulfur-bridged diiron cluster that is connected to an iron−sulfur cubane, while the [NiFe] hydrogenase's active site contains a dinuclear iron−nickel cluster.

Here, we focus on a structural and electronic comparison of [FeFe] hydrogenase and Hmd. At first sight, both active sites could not be more different. However, the point to make in this work is that a closer inspection of the reaction center, which is in both cases a single iron center, reveals rather similar first-shell ligand environments.

Unifying Structural−Electronic Concept

First of all, in the trans position to the reacting ligand, i.e., trans to molecular hydrogen[1d,3a,4] or dioxygen in an inhibition reaction,[5] we find a carbon atom of a C═O moiety. This sp^2 carbon atom is in Hmd also bound to another carbon atom of the organic chelate ligand, while it is in [FeFe]

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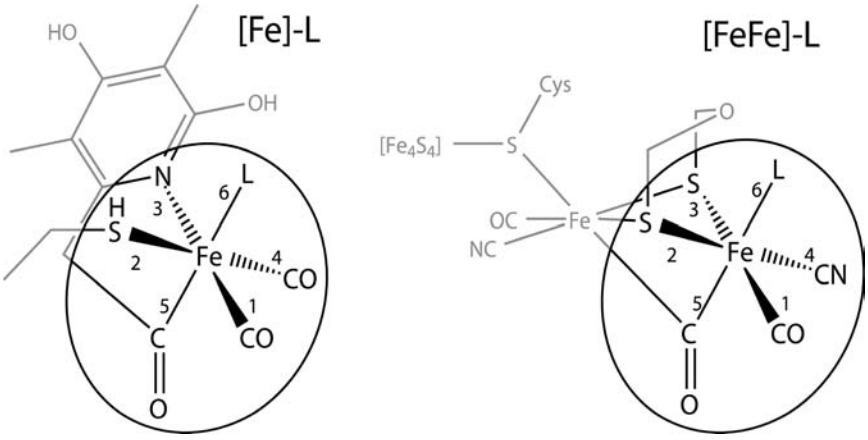

Figure 1. Lewis structures of the active sites of Hmd and [FeFe] hydrogenases {[Fe]-L (left) and [FeFe]-L (right), respectively} depicted such that the similar ligand environments of the reacting site are highlighted.

hydrogenase bound to the second iron atom of the $[2Fe]_H$ (sub)cluster. Formally, the acyl ligand carries a negative charge, whereas the bridging CO ligand can be considered uncharged. However, although such a *formal* charge assignment is useful for classification purposes, it has no direct counterpart in quantum mechanics and does not require much attention in this first approach toward a unifying structural and electronic concept.

Next, the remaining four ligands form a plane that we may call the equatorial plane. In this plane, we find two σ donors on one side opposed by two strong π acceptors (CN^- and CO) on the other side. For a sketch of the first ligand spheres of the active iron atoms in the active sites, see Figure 1, which also introduces a short-hand notation and a ligand numbering scheme.

Next, in our gedankenexperiment, we ignore the second iron atom in the dinuclear active site of [FeFe] hydrogenase and assign to it a predominantly structural role in order to provide the specific ligand environment for the catalytic iron center (namely, two sulfur σ-donor atoms plus a bridging carbonyl ligand). One might argue that spin−spin interactions of the two iron atoms may play a role but the iron atoms are in strong ligand environments (even if antiferromagnetic coupling would take place, the energetic contribution is negligible compared to coordination energies).

Our assumption thus is that it is the *constitution of ligands of the same type around an octahedral iron atom that determines the reactivity* with respect to the activation and processing of dihydrogen. Hence, we stipulate that a dihydrogen-converting iron atom should feature some kind of bridging CO ligand in the trans position to the reacting ligand. Then, two strong ligands are required in positions 1 and 4, i.e., in a cis orientation opposite to two σ donors that then build the equatorial plane of the coordination octahedron. Note that this working hypothesis holds for hydrogenases relying on a single reacting iron center, which does not hold for [NiFe] hydrogenase because in this enzyme nickel plays a significant role.

If organizations of the coordination environments of the reacting iron centers in [FeFe] hydrogenase and Hmd, respectively, are indeed similar, we should be able to observe similar reactivities if the ligand environments are swapped; i.e., the energetics of reactions at the iron center should be unchanged if the reacting iron atom of [FeFe] hydrogenase is placed in the first ligand sphere of Hmd and all of the other structural elements of the [FeFe] active site are left unchanged and vice versa. Thus, we may swap the ligand atoms in the first coordination sphere of the iron center without changing the very nature of the active site (i.e., the overall cluster structure). It is, in principle, easy to change the strong ligand CO for the strong ligand CN^-, but to swap the σ-donating ligands is somewhat more involved. The acyl ligand does not require any changes at all because this moiety has its counterpart in the bridging CO ligand of the dinuclear iron cluster in [FeFe] hydrogenase.

In the case of [FeFe] hydrogenase, we need to change one sulfur atom in the dithiolate bridge to a nitrogen atom and have to decide whether we insert an imine-derived ligand with a double bond to the carbon atom (like in the pyridine blueprint of Hmd) or an amide with additional hydrogen atoms bound to the nitrogen atom featuring a single bond to the carbon atom. The swapped ligand environment with the imine-like bridge is denoted [FeFe]**1**-L, while the amide-bridge-containing system is called [FeFe]**2**-L. Now, sulfur is a group 6 element, while nitrogen is of group 5. In order to account for this in Hmd, we choose thiabenzene and thiopyrane derivatives and introduce the swapped ligand spheres **1** for the former and **2** for the latter denoted as [Fe]**1**-L and [Fe]**2**-L, respectively. This also means that we change the overall structure of the chelate ligand in Hmd as little as possible. The resulting swapped ligand spheres are depicted in Figure 2.

So far, these are only assumptions that might be difficult to test in experiment. Here, quantum chemical calculations come into play, in which we may easily swap the ligand environments, optimize the resulting structures, and compare ligand coordination energies with those in the wild-type ligand arrangement given in Figure 1. Note that the [FeFe]-L site features a rich redox chemistry compared to Hmd, [Fe]-L, whose most favored oxidation state should be low-spin iron(II) according to a spectroscopic study of Wang et al.[6] On the contrary, [FeFe] hydrogenase exists in two different redox states during its catalytic cycle, namely, an electron paramagnetic resonance (EPR)-silent form that formally corresponds to a $[Fe^IFe^I]$ subsite and an EPR-active $[Fe^IFe^{II}]$ species. In a further oxidized $[Fe^{II}Fe^{II}]$ state, the enzyme is inactive but can be reactivated under reducing conditions.[15−18] In [FeFe] hydrogenase, the active species is likely to be in the

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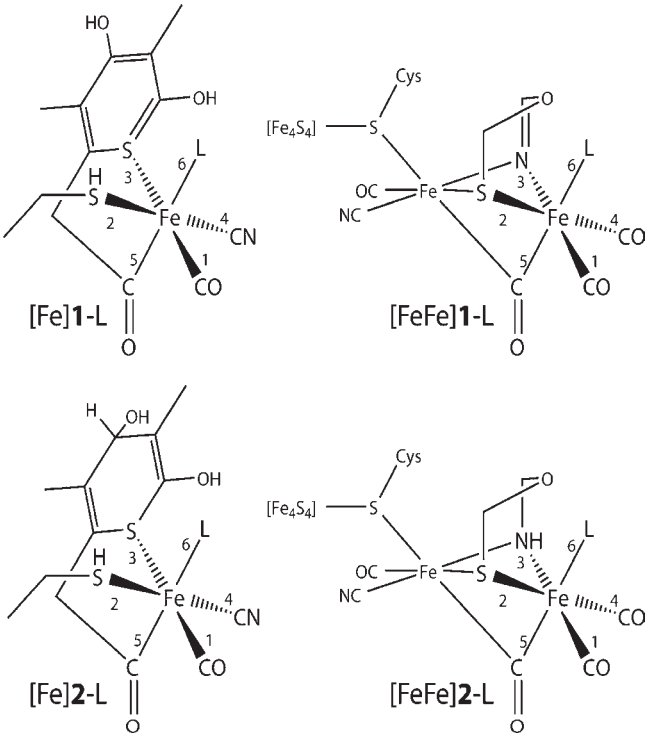

Figure 2. Lewis structures of the active sites of [FeFe] and Hmd hydrogenases in *swapped* coordination environments. Note that these models must be differently charged in order to present the reactive iron atom in the formal oxidation state II+. In particular, if L is an uncharged ligand, then [Fe]**1**-L carries one positive charge and [Fe]**2**-L is uncharged, while both [FeFe] sites in swapped ligand environments are negatively charged.

$Fe^{I}Fe^{II}$ oxidation state.[1b,c] In this first presentation of the unifying structural−electronic concept, we shall focus on reacting iron centers in the oxidation state II+ in all complexes under consideration here. However, we should note that future work needs to address how the different atoms of the first-shell ligands facilitate a change of the oxidation state in [FeFe] but not in Hmd hydrogenases despite the very same nature of these ligand atoms in both active sites.

Quantum Chemical Methodology

All-electron calculations were carried out with the density functional theory programs provided by the *Turbomole* suite[7] on model structures of the active sites. In the case of [FeFe] hydrogenase, we take the proximate Fe_4S_4 cluster explicitly into account. The models were treated as open-shell systems in the unrestricted Kohn−Sham framework. For the calculations, we used the Becke−Perdew exchange-correlation functional dubbed BP86[8,9] and we invoked the resolution-of-the-identity (RI) approximation as implemented in *Turbomole*. For all atoms included in our models, we used Ahlrichs' valence triple-ζ TZVP basis set with polarization functions.[10] All molecular structures were fully optimized in unrestricted Kohn−Sham calculations in their low-spin state. Note, in particular, that we have not considered the thiomethanolate residues to be spatially fixed in order not to distort the structure because of assumptions made for the [FeFe] protein environment. The basis set superposition error[11] of the TZVP basis set with respect to coordination energies is below 3.0 kcal/mol and will, therefore, not affect analysis of the coordination energies. For instance, the coordination-energy counterpoise correction[12] for water coordinated to the Hmd active site model yields 2.2 kcal/mol, while it is only 0.7 kcal/mol for coordination of dihydrogen to the same model active site. All reaction energies given in the manuscript have not been corrected for such basis set superposition effects and have been evaluated at 0 K without vibrational and temperature corrections. Pictures of the molecular structures were created with *PyMOL*.[13]

Naturally, we have to choose model clusters of the appropriate size to model the enzymatic active sites. For [FeFe] hydrogenase, we employ the well-established cluster structures according to refs 4k, 5d, and 14. In the case of Hmd, we focus on the metal cluster with coordinates taken from the 1.75 Å crystal structure of methylenetetrahydromethanopterin dehydrogenase from *Methanocaldococcus jannashii* (PDB entry 3F47)[2d] and follow the model approach by Yang and Hall,[3b] with one modification however: the methyl group at the sulfur atom modeling a cysteine residue was substituted by an ethyl residue (also the cysteine residue is protonated in order to provide a closer analogy to the thiolate bridges in the [FeFe] site, where each sulfur atom is also bound to three other atoms).

In order to judge the amount of intramolecular reorganization upon ligand binding, we compare reaction energies obtained from electronic energies for optimized structures of the ligand-bound structure ($E_{\mathrm{cluster-ligand,relaxed}}$), the free structure ($E_{\mathrm{cluster,relaxed}}$), and the ligand structure ($E_{\mathrm{ligand,relaxed}}$)

$$D_{\mathrm{e}} = E_{\mathrm{cluster-ligand,\,relaxed}} - E_{\mathrm{cluster,\,relaxed}} - E_{\mathrm{ligand,\,relaxed}}$$

with intrinsic coordination energies calculated from the electronic energy of the structure-optimized ligand-bound complexes and single-point energies for the sixth-ligand-free cluster ($E_{\mathrm{cluster,frozen}}$) and ligand ($E_{\mathrm{ligand,frozen}}$) taking the coordinates from the optimized ligand-bound complex structure

$$D_{\mathrm{e}}^{\mathrm{int}} = E_{\mathrm{cluster-ligand,\,relaxed}} - E_{\mathrm{cluster,\,frozen}} - E_{\mathrm{ligand,\,frozen}}$$

Results and Discussion

Structure optimization for the complexes in Figures 1 and 2 with L = H_2O and H_2 yields a first important result, namely, that the swapped ligand environments produce stable complexes that neither largely rearrange nor decompose.

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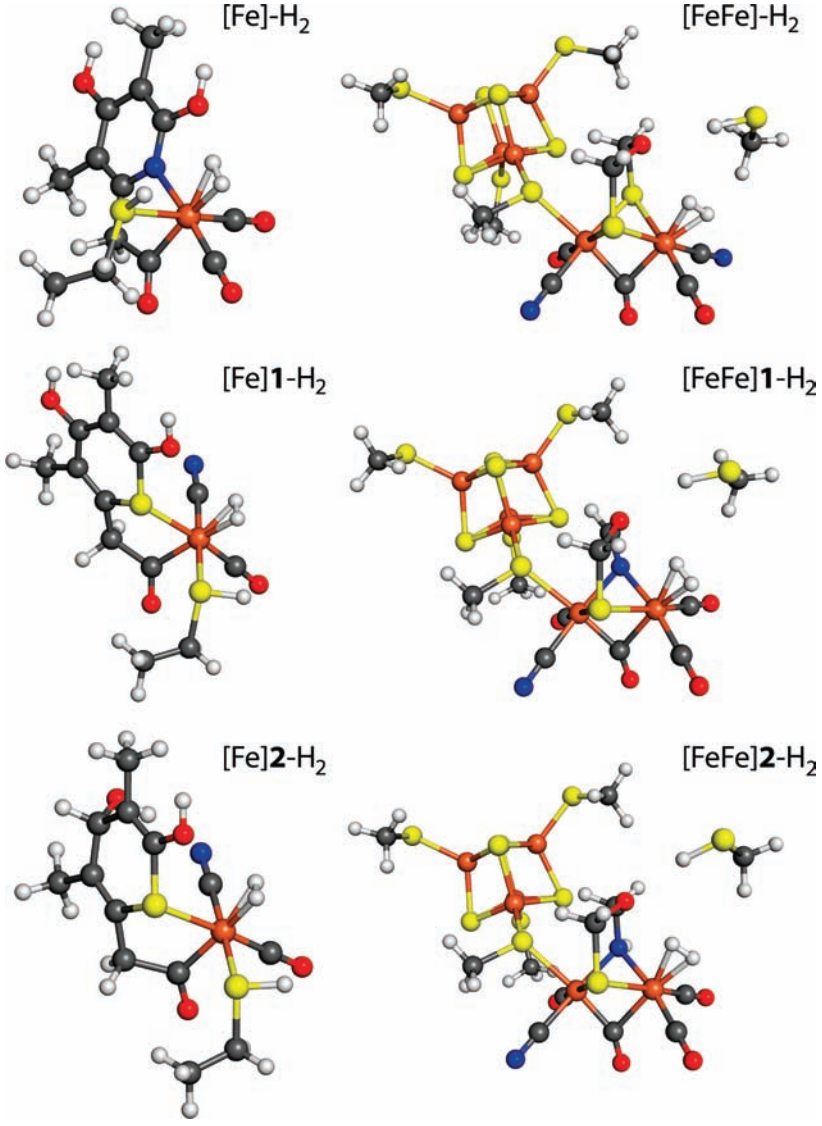

Figure 3. BP86/RI/TZVP-optimized structures of the active sites of [FeFe] and Hmd hydrogenases in original and *swapped* coordination environments with a coordinating dihydrogen ligand: (top-left) [Fe]-H_2, (middle-left) [Fe]**1**-H_2, (bottom-left) [Fe]**2**-H_2, (top-right) [FeFe]-H_2, (middle-right) [FeFe]**1**-H_2, (bottom-right) [FeFe]**2**-H_2. Note that all structures exactly resemble the structures of Figure 2, although some have been rotated for visibility reasons. Element coloring scheme: C, gray; Fe, brown; H, white; N, blue; O, red; S, yellow.

Moreover, their structures are very similar (cf. the structures of the dihydrogen complexes in Figure 3).

Next, we compare coordination of water to the native active sites found in X-ray structures[2b,d,14] and to their analogues in swapped ligand spheres. If the concept of similar ligand environments elaborated here is meaningful, we would expect to find similar coordination energies for native and swapped ligands. Of course, one should not expect them to be identical. We found in our calculations that the coordination energy D_e of water is −13.3 kcal/mol in [Fe], which is very close to those in swapped ligand spheres: −11.9 and −16.3 kcal/mol for [Fe]**1**-H_2O and [Fe]**2**-H_2O, respectively. Coordination of water to the [FeFe] fragments features very similar energies that are also comparable to those of the Hmd structures. The native ligand spheres of [FeFe] coordinates water by −6.3 kcal/mol, while the swapped ligand spheres of [FeFe]**1** and [FeFe]**2** liberate a similar amount of energy, namely, −4.5 and −5.3 kcal/mol, respectively.

However, the energies are not sufficient to establish an analogy between native and swapped ligand environments. The molecular structures need to be investigated in detail as well. In fact, water is very weakly coordinated to the iron atom of the three Hmd-type species considered and is held in position 6 by a strong hydrogen bond to the phenol hydrogen atom in close proximity. For comparison, the BP86/RI/TZVP hydrogen bond energy in Ph−OH···H_2O amounts to −7.2 kcal/mol, and we may thus attribute about half of the −13.3 kcal/mol coordination energy to such a hydrogen bond. Figure 4 shows the hydrogen-bonding network of the three Hmd species considered and suggests that in each case hydrogen bonding plays a significant role for water coordination.

A close contact between the water oxygen atom and the central iron atom can only be observed for [Fe] and [Fe]**2**, whereas in [Fe]**1**, the water molecule is solely bound by hydrogen bonds. Here, a proton transfer took place upon structure optimization, where one proton from the water molecule was moved to the coordinating CN^- group and was replaced by the proton of the OH group of the thiobenzene moiety. Also, in [Fe] and [Fe]**2**, hydrogen bonding strongly

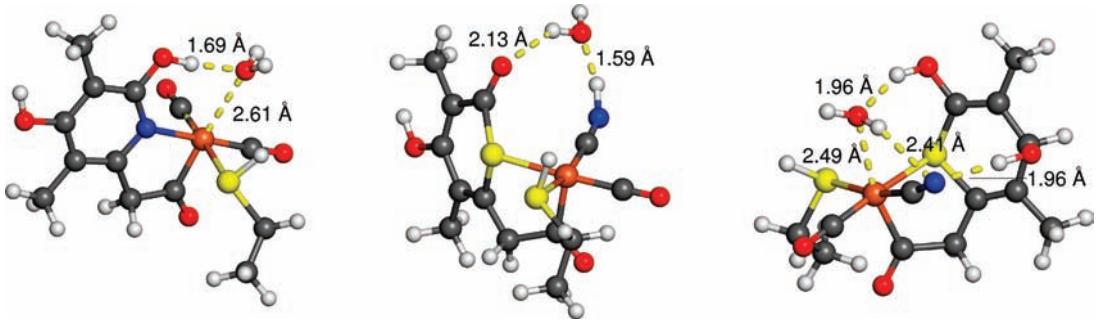

Figure 4. Hydrogen-bonding network in the BP86/RI/TZVP-optimized structures of Hmd hydrogenase in unmodified and swapped coordination environments: (left) [Fe]-H_2O; (middle) [Fe]**1**-H_2O; (right) [Fe]**2**-H_2O. The view is adapted for each compound for visibility reasons. Bond distances are given in angstroms.

Table 1. Collection of BP86/RI/TZVP Reaction Energies D_e and Intrinsic Coordination Energies D_e^{int} (i.e., Calculated with Unrelaxed Fragments) for the Addition of Water and Dihydrogen to the Active Site of Hmd and [FeFe] Hydrogenases in the [$Fe^{II}Fe^{II}$] Redox State in a Wild-Type Configuration and with Swapped Ligand Environments (in kcal/mol)

	Hmd hydrogenase					
	wild type		[Fe]**1**		[Fe]**2**	
	D_e	D_e^{int}	D_e	D_e^{int}	D_e	D_e^{int}
H_2O	−13.3	−17.0	−11.9	−16.6	−16.3	−17.0
H_2	−5.6	−34.4	18.7	−13.3	−8.3	−11.1
	[FeFe] hydrogenase					
	wild type		[FeFe]**1**		[FeFe]**2**	
	D_e	D_e^{int}	D_e	D_e^{int}	D_e	D_e^{int}
H_2O	−6.3	−11.0	−4.5	−8.3	−5.3	−13.1
H_2	−3.7	−12.9	3.7	−5.3	3.8	−4.7

contributes to water coordination. In both compounds, the phenol group of the ligand forms a hydrogen bond with the oxygen atom of water.

The additional hydrogen bond between one water hydrogen atom and the nitrogen atom of the CN^- ligand could explain the higher exothermicity of water coordination to [Fe]**2**.

Hence, all three variants of Hmd weakly coordinate water and hold it in position 6 via strong hydrogen bonds to acceptors and donors in the ligand sphere. In the case of [FeFe] hydrogenase, coordination of water to the distal iron atom of the 2$[Fe]_H$ subsite is only assisted by a weak hydrogen bond between a water hydrogen atom and the oxygen atom of the bridging dithiolate group (not shown).

When we now investigate coordination of dihydrogen, we obtain six coordination energies in a quite narrow range between −5.6 and +18.7 kcal/mol, although three of them are endothermic (see Table 1). However, this is because the metal fragments distort in different ways upon coordination. In [Fe]-H_2, the structural relaxation of the fragment [Fe] yields −27.3 kcal/mol and the intrinsic dissociation energy defined for *unrelaxed* fragments after dissociation of the unrelaxed ligand is −34.4 kcal/mol.

The intrinsic coordination energies for the two swapped Hmd models are similar, −13.3 kcal/mol for [Fe]**1**-H_2 and −11.1 kcal/mol for [Fe]**2**-H_2, and less exothermic than in the case of the native system (−34.4 kcal/mol).

The intrinsic coordination energies for the native and swapped [FeFe] models in Table 1 are very similar for both coordination reactions. For [FeFe] hydrogenase variants,

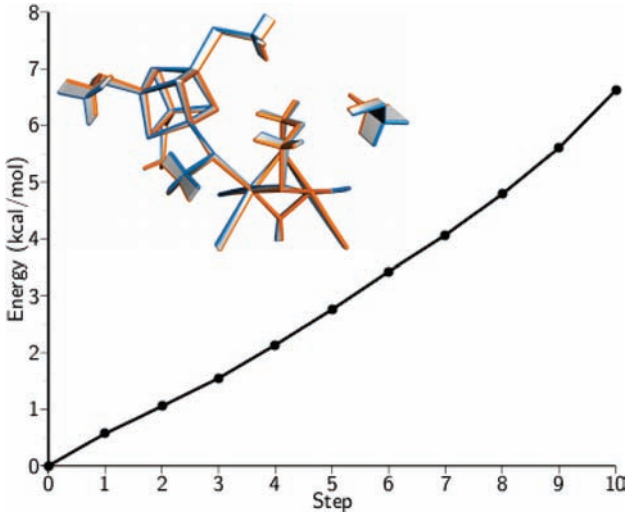

Figure 5. Energy change obtained for a stepwise linear transition from the relaxed ligand free structure of [FeFe] hydrogenase (blue) to the structure of the fragment in the dihydrogen-bound complex (orange). The vector distance between the two structures was taken and properly scaled to obtain nine intermediate coordinate sets, for which BP86/RI/TZVP single-point calculations were performed.

we observe consistently a difference between intrinsic and regular coordination energies of approximately 10 kcal/mol. In order to better understand the amount of energy that is invested in structural reorganization upon dihydrogen binding, we performed a stepwise linear transition from the relaxed ligand-free cluster toward the fragment structure taken from the dihydrogen-bound complex and performed single-point calculations for each intermediate (Figure 5).

Clearly, this transformation leads to a continuous increase of the molecular energy by about 6.6 kcal/mol in total, which then reduces the intrinsic coordination energy to the (observable) coordination energy.

Modulation of the Electronic Structure via Ligand Variance

We have elaborated on the structural and, hence, electronic similarities of [FeFe] and Hmd's active sites. Of course, the explicit differences in the ligand environment finally result in a modulation of reactivity, despite the qualitative similarities discussed so far, and we shall discuss the most pronounced effects in this section. The reactivity of the clusters is not identical, and the use of different ligands by nature must have a detectable effect. For instance, there should be a measurable difference between the conserved CN^- ligand in [FeFe]

hydrogenases and the corresponding CO ligand in Hmd. De Gioia and co-workers[19] investigated this particular issue by analyzing the consequences of an exchange of the CN^- ligands in [FeFe] hydrogenase against CO in terms of a molecular orbital diagram and found a role in the fine-tuning of the electronic and redox properties of the enzyme. This affects protonation chemistry as well as electron transfer between the cubane and the $[2Fe]_H$ subsite.[19] It was also suggested that for the protein-embedded cluster the CN^- ligand at the distal iron center of the $[2Fe]_H$ subsite may have a significant mechanistic impact on catalysis because it favors the formation of a terminal hydrid adduct over a μ-bridged isomer by forming a salt bridge with a nearby lysine group.[20]

In order to establish the structural similarities between both active sites, we considered the cysteine sulfur atom of the Hmd species to be in a protonated state. Interestingly, deprotonation leads to an increase in the endothermicity for coordination of dihydrogen, yielding total coordination energies of 5.1 kcal/mol for [Fe], 27.2 kcal/mol for [Fe]**1**, and 3.8 kcal/mol for [Fe]**2**. In comparison, the addition of water becomes slightly more exothermic, namely, −8.1 kcal/mol for [Fe], −11.0 kcal/mol for [Fe]**1**, and −18.3 kcal/mol for [Fe]**2**.

Furthermore, regarding the similarity between the bridging CO ligand of [FeFe] hydrogenase and the acyl ligand of Hmd, one must keep in mind that the latter is sterically much more restricted and cannot undergo conformational changes during the catalytic cycle, unlike the small CO ligand does, which switches between a μ-bridging and a terminal position.

Also, the overall reaction mechanisms for hydrogen production/oxidation differ between Hmd and [FeFe] hydrogenases. Whereas in the case of the latter the electron transfer to/from the $[2Fe]_H$ subsite is facilitated by a number of auxiliary FeS clusters and the reaction takes place by the direct protonation and reduction at the distal iron atom, the active center of Hmd interacts with the cofactor MPT^+ (N^5,N^{10}-methenyltetrahydromethanopterin), which acts as a hydride donor/acceptor.[3] The different ligand compositions observed in [FeFe] and Hmd hydrogenase could therefore be an adaption to distinct mechanistic requirements regarding interaction with cofactors and electron transfer rather than affecting the overall energetics of ligand binding to the active sites. This is in line with our observation that swapping the ligand environments in Hmd and [FeFe] hydrogenase does not have a strong impact on the coordination energies of the natural ligands water and dihydrogen.

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Conclusion

To conclude, we have presented a design principle for Hmd and [FeFe] hydrogenase active sites that is based on an *octahedral iron atom surrounded by ligands of the same type and in the same position that determines reactivity*. As a consequence, it was possible to design swapped ligand spheres for each of the active sites. A first important result of the structure optimization of these complexes was that these swapped ligand spheres produce stable complexes.

In view of the strikingly similar reaction energies for [FeFe] and Hmd hydrogenases with wild-type and swapped ligands, we obtain a hint pointing at a general principle for ligand selection for dihydrogen reactions at single iron centers. In future work, we shall address the thermochemistry of a consecutive exchange of ligands as well as of additional steps in the reaction mechanism. Moreover, the effect of a swapped ligand environment on barrier heights, transition-state structures, and charge distributions at the active iron site shall be investigated. This new principle leads us to ligand design aspects of hydrogenase chemistry as well as to new design principles for biomimetic model chemistries.

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