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Oxygen Coordination to the Active Site of Hmd in Relation to [FeFe] Hydrogenase

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The application of [FeFe] hydrogenases for renewable energy production is limited by the generally high O_2-sensitivity of this class of enzymes. In this work, we report energy differences in oxygen coordination at the active sites of [FeFe] and monoiron (Hmd) hydrogenase as obtained from DFT calculations. Whereas O_2 addition is clearly exothermic in the case of [FeFe] hydrogenase, it is endothermic for monoiron hydrogenase. By applying a recently proposed concept [*Inorg. Chem.* **2010**, *49*, 5818], which allows us to structurally and electronically relate the two active sites, we show that an inversion in oxygen affinity is observed as a result of mutually exchanging the first ligand sphere of the central iron atoms of both catalytic centers. Modified Hmd active sites can bind O_2 exothermically, whereas O_2 addition to the altered H-cluster variants is less exothermic than in the wild-type configuration. Our results show that oxygen affinity is not very strong when measured in terms of the coordination energy. Hence, changes in the first ligand shell of the reactive iron atoms with retention of the similarity of the ligand spheres, that is, having the same type of ligands in the same positions of the coordination octahedron, can render oxygen coordination endo- or exothermic. This observation could explain the difference in oxygen sensitivity of Hmd and [FeFe] hydrogenases.

1. Introduction

Hydrogenases catalyze either the formation or the oxidation of molecular hydrogen[1–5] and are thus important biological reaction systems for clean energy technologies (fuel cells, hydrogen production).[6–8] A major difficulty for the application of hydrogenases in technological processes is their irreversible inactivation by molecular oxygen.

On the one hand, it has been found that the active site of [FeFe] hydrogenase, which is particularly efficient in the production of H_2, decomposes after coordination of O_2, as has been elaborated in recent experimental[9–11] as well as in theoretical[12,13] work. On the other hand, it is very intriguing that the mononuclear methylenetetrahydromethanopterin dehydrogenase (Hmd) shows no sensitivity against molecular oxygen. Although oxygen may affect various parts of the enzyme, the former observation suggests that the reactivity of the active sites of these enzymes towards oxygen can give decisive insight into the oxygen sensitivity problem. For this reason, we consider the coordination chemistry of triplet dioxygen to generic cluster models of these active sites as the essential first step to study. We pursue the hypothesis that O_2 binding to the reactive metal centers is a prerequisite for cluster degradation, as proposed for [FeFe] hydrogenase on the basis of experimental[9,10] and theoretical studies.[12] Only when dioxygen is sufficiently strongly bound to the catalytic center can subsequent reaction steps decompose and thus inactivate this site.

Here we investigate whether the insensitivity of Hmd against dioxygen can be explained by the coordination energetics of O_2 binding to its active site. We employ density functional theory (DFT) and consider structural models of the active site only. Apart from directly coordinating amino acid residues, the protein environment is not considered in these calculations. Although one may view this as a drawback, as conclusions are drawn on the basis of a small structural model, it actually allows us to determine the generic coordination chemistry of the active sites, which is considered to be then modulated by the protein environment. We also consider this approach as the first step towards an understanding of the coordination energetics in the complete enzyme, for which different structures may become relevant (wild-types from different organisms or enzymes obtained from mutagenesis studies).

Moreover, by applying a recently proposed unifying structural and electronic concept for the active sites of Hmd and [FeFe] hydrogenase,[14] we elucidate the influence of the particular ligand compositions on dioxygen coordination. This is of interest for further investigations that will address the central question in this context, namely whether the technologically interesting [FeFe] hydrogenase might be modified such that it is less sensitive to an attack of molecular oxygen at the active site. For this purpose, one option might be redesigning the first-shell ligand environment of the catalytically active metal center, that is, of the distal iron

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center in the dinuclear iron cluster of [FeFe] hydrogenase. Even if this might lead to ligand environments that cannot be synthesized in vivo, it will provide hints for the design of functional biomimetic model complexes or for mutagenesis studies that change the distant ligand environment.

2. Computational Methodology

All-electron unrestricted Kohn–Sham calculations were carried out with the DFT programs included in the Turbomole 5.10 suite of programs[15] on model structures of the active site of [FeFe] hydrogenase by taking the proximate Fe_4S_4 cluster explicitly into account (for details, see discussion below). 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[16,17] unless otherwise noted and invoked the resolution-of-the-identity (RI) approximation as implemented in Turbomole. For selected structures we have conducted comparisons with the TPSS functional,[18] the B3LYP hybrid functional,[19,20] and the B3LYP* functional.[21–23] For all atoms included in our models, we used Ahlrichs' valence triple-ζ TZVP basis set with polarization functions.[24] Predefined spin distributions were screened with our restrained local-spin optimization tool.[25,26]

Since we consider charged species, care must be taken if reactions involving (1) differently charged species or (2) charge separations are compared. In order to assess the effect of charge stabilization by the environment (i.e., the protein), we carried out calculations with an electrostatic continuum model. For this purpose we chose the COSMO approach[27] as implemented in Turbomole. For additional information on the computational methodology we refer the reader to the Supporting Information.

3. Oxygen Coordination to the Active Sites of [FeFe] and Hmd Hydrogenase

The structure of Hmd's active site has been elucidated with nuclear resonance and vibrational spectroscopy as well as X-ray crystallography,[28–31] which was a prerequisite for first quantum chemical investigations into its reaction mechanism.[32,33] We follow the modeling approach of Hall and co-workers and employ their active site model but use a thioethyl instead of a thiomethyl ligand to mimic the cysteine residue. For the calculations on this Hmd model, we considered the formal (low-spin) oxidation state +II for the central Fe atom in line with the spectroscopic study of Wang et al.[34] To study the active site of [FeFe] hydrogenase (the H-cluster), we follow our previous approach, which considers the $2[Fe]_H$ subsite and the auxiliary $[Fe_4S_4]$ cubane as well as a neighboring cysteine residue that may play a role in proton transfer reactions.[12] If not otherwise mentioned, we consider the active oxidized H_{ox}^{cat} form of the H-cluster, which corresponds to a doublet spin state.[3] In order to complement our recent data on [FeFe] hydrogenase, we use here a molecular model of the active site that better captures the restrained flexibility available to the H-cluster in its protein environment by spatially fixing the carbon atoms of the thiomethanolate and thiomethane groups as well as one hydrogen atom that corresponds to the C_α atom of the particular cysteine residue under consideration. Thereby we can model the rotameric degree of freedom available to a cysteine side chain. Regarding the ambiguity of the bridgehead group of the dithiolate ligand, we compare the possibilities of an O atom or a NH group at that position.

In order to elucidate the difference in dioxygen sensitivity of [FeFe] and Hmd hydrogenase, we follow our recently proposed approach and study the stability of the active sites with respect to coordination of molecular oxygen, which can be considered as the first step in a potential degradation cascade of reactions that may eventually decompose the metal clusters as was shown to be a viable reaction pathway for [FeFe] hydrogenase.[12,13] For this purpose, we investigate the coordination energy of triplet O_2 attacking the metal fragment active site of Hmd [Equation (1)]:

$$^{1}[\mathrm{Fe}] + {}^{3}\mathrm{O}_2 \rightarrow [\mathrm{Fe}](\mathrm{O}_2), \tag{1}$$

or of [FeFe] hydrogenase, respectively [Equation (2)],

$$[\mathrm{FeFe}] + {}^{3}\mathrm{O}_2 \rightarrow [\mathrm{FeFe}](\mathrm{O}_2) \tag{2}$$

We consider the exothermicity of this process as a prerequisite for subsequent cluster degradation.[12] Note that in the case of [FeFe] hydrogenase we focus here on the active oxidized H_{ox}^{cat} form of the H-cluster.

From the O_2 coordination energies given in Table 1 it is clear that O_2 addition to the active oxidized H_{ox}^{cat} form ([FeFe]) is exothermic for all considered binding modes of O_2 (compare Figure 1). The different treatment of the cluster fixation results in an increased exothermicity for the formation of the terminal O_2 adduct compared to our previous work,[12,35] where we considered the methyl groups to be spatially completely fixed. With O and NH in the bridge-

Table 1. Reaction energies for the formation of O_2 adducts of the considered [FeFe] hydrogenase variants (H_{ox}^{cat} form) shown in Figure 5. Top: NH in bridgehead position. Bottom: O in bridgehead position. Energies are given in kcal/mol. The second numbers in column 2 refer to coordination energies obtained by employing the COSMO approach to account for solvent effects ($\varepsilon = 4.0$), which we provide for the experimentally important NH group in the bridgehead and terminal binding mode only.

Educt		O_2 binding mode	
	Terminal	μ-Bridged	Linear bridged
$[FeFe]^{3-}$	–16.9/–17.5	–7.4	–17.2
[FeFe]**1**$^{2-}$	–10.5/–10.0	6.1	0.6
[FeFe]**2**$^{2-}$	–12.1/–13.5	10.0	3.3
[FeFe]**3**$^{2-}$	–10.7/–10.6		
[FeFe]**4**$^{3-}$	–12.5/–13.7		
$[FeFe]^{3-}$	–15.2	–9.6	–23.5
[FeFe]**1**$^{2-}$	–9.8	2.7	–8.7
[FeFe]**2**$^{2-}$	–10.5	4.7	–2.2
[FeFe]**3**$^{2-}$	–8.8		
[FeFe]**4**$^{3-}$	–14.2		

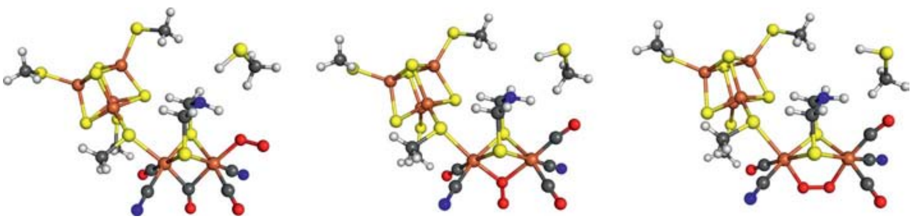

Figure 1. Terminal (left), μ-bridged (middle), and linear bridged (right) O_2 adduct of the H-cluster of [FeFe] hydrogenase with NH as in the bridgehead position. White: H, red: O, gray: C, blue: N, yellow: S, brown: Fe.

head position, we obtain very similar results of –16.9 and –15.2 kcal/mol (Table 1, [FeFe]). However, recent experimental and theoretical findings are in strong favor of NH in the bridgehead position of the dithiolate ligand, and we therefore focus on this choice.[41,42]

With the NH-bridgehead group, the linear bridged and the terminal oxygen adduct are equally favored, whereas the latter is the most likely one formed in terms of accessibility from the gas-diffusion channel, given the cluster conformation observed in the published crystal structures.[36–38] These data again show that the coordination of O_2 to the distal Fe_d atom of the H-cluster is an exothermic process.

The exothermicity of oxygen coordination is confirmed by structure optimizations with other density functionals: –19.1 kcal/mol with TPSS, –9.2 kcal/mol with B3LYP (20% Hartree–Fock exchange), and –12.0 kcal/mol with B3LYP* (15% Hartree–Fock exchange; single-point calculation on B3LYP structures). When we now study the situation for the active site of Hmd, we find that the central iron atom does *not* coordinate triplet dioxygen. The coordination energy is endothermic by +9.6 kcal/mol (BP86, see Table 2), in sharp contrast to that for [FeFe] hydrogenase. Hence, the first step of an inhibition and eventually gradual degradation process is not likely to occur. Note, however, that the endothermicity is a result of two-state reactivity,[39] as the O_2 complex is endothermic relative to triplet oxygen and the metal fragment, but the complex can be optimized on the singlet hypersurface as it is stable on the singlet potential energy surface.

Table 2. Dioxygen coordination energies for the considered Hmd variants in kcal/mol. The second numbers refer to coordination energies obtained by employing the COSMO approach to account for solvent effects ($\varepsilon = 4.0$). The charges given refer to the protonated and deprotonated form of the cysteine residue, respectively. Note that we do not report COSMO results for [Fe]**3**$^{+/0}$ and [Fe]**5**$^{0/-}$. While it is sufficient to discuss the isomer [Fe]**5b**$^{0/-}$ in the latter case, we observed in the former case that continuum solvation favors a structure in which no proton transfer is observed, and this result can thus not be directly compared with the one for the isolated ("gas-phase") species where a proton is transferred from the phenol OH group to the cyanide ligand. The non-observed proton transfer in the COSMO calculation on [Fe]**3**$^{+/0}$ results in an *exothermic* coordination energy, as one would expect on the basis of the swapped-ligands model.

	Coordination energy [kcal/mol]	
Oxygen adducts	Protonated Cys-S	Deprotonated Cys-S
[Fe]$^{+/0}$	6.4/10.5	9.6/9.1
[Fe]**1**$^{0/-}$	–1.3/0.5	–8.4/–6.9
[Fe]**2**$^{2+/+}$	3.9/10.5	3.1/3.9
[Fe]**3**$^{+/0}$	9.5/–	11.9/–
[Fe]**4**$^{+/0}$	7.6/8.3	4.9/5.6
[Fe]**5**$^{0/-}$	–6.6/–	–10.4/–
[Fe]**5b**$^{0/-}$	–1.1/–0.9	–4.4/–6.9

4. Influence of Ligand Composition on Dioxygen Sensitivity

Now, after having established that the endothermic coordination of triplet oxygen can prevent Hmd from being inhibited, we may ask how the particular ligand compositions of the two hydrogenases influence the energetics of dioxygen binding to the central iron atoms of their active sites. In this context, it is of special interest whether ligand design might allow to prohibit that the active site of [FeFe] hydrogenase is attacked and then decomposed by O_2.[12] In order to investigate these questions, we use and extend the principle of "swapped ligand spheres", which we have recently established[14] and which allows us to structurally and electronically relate Hmd and [FeFe] hydrogenases. This principle utilizes the fact that the first ligand shell of the reactive iron center in both hydrogenases is similar in terms of ligand types and coordination positions (see also Supporting Information). We proceed here in the very same way as in ref.[14] (see Figures 2 and 3). In case of Hmd, we extend the design approach[14] in the present work by changing the wild-type configuration *stepwise* into the two swapped architectures that resemble the coordination chemistry of the Fe_d atom in [FeFe] hydrogenase. As a result, six different molecular species emerge (see Figure 2). Note that in order to establish identical connectivities for the atoms involved in Fe coordination between Hmd and [FeFe] hydrogenase, we choose the sulfur atom of the coordinating cysteine residue in Hmd to be protonated but we also compare with the deprotonated form when we study dioxygen coordination. Additionally, we also consider single substitutions of the CN^- ligand at the distal Fe_d atom of the $[2Fe]_H$ subsite, namely by CO and by Cl^- in order to study the role of a strong and charged π-acceptor (CN^-) at ligand position 4 (Figure 2).

As a first important result, it must be noted that all considered variants of the active sites of [FeFe] hydrogenase and Hmd form stable complexes that do not undergo decomposition during structure optimization, neither in free nor in O_2-bound state (Figures 4 and 5). A discussion of the subtle changes in molecular structure is given in the Supporting Information.

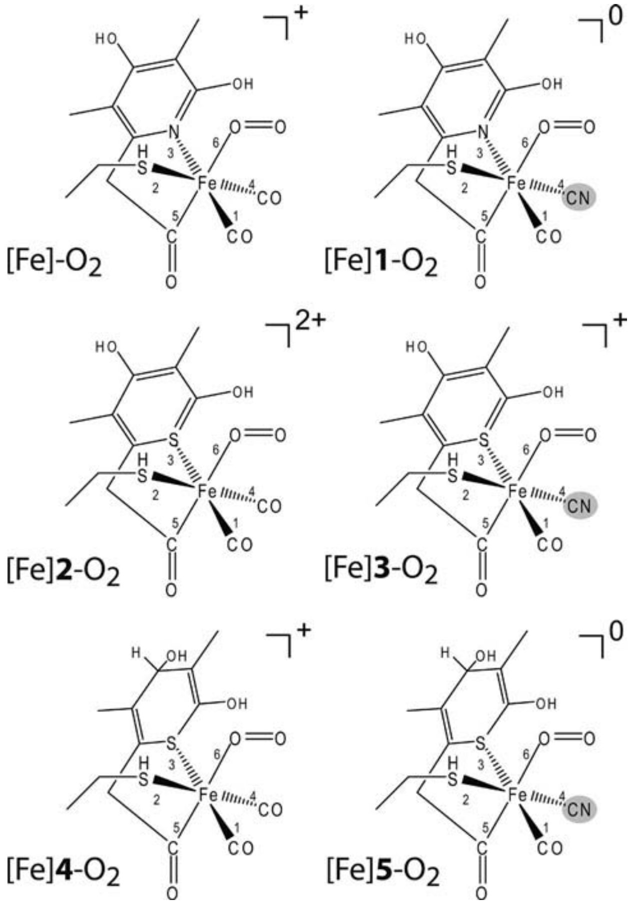

Figure 2. Lewis structures of dioxygen adducts of the Hmd variants in different ligand environments. Note that the given charges correspond to the protonated form of the cysteine ligand (ligand position 2). Upon deprotonation, these charges have to be reduced by one elementary charge. All complexes are considered in an open-shell singlet spin state. Shaded ovals highlight substitutions at ligand position 4.

The coordination energies listed in Table 2 show that the endothermicity of O_2 addition to the Hmd variants depends on the protonation state of the coordinating cysteine sulfur atom. For the native active site, the binding energy drops from 9.6 to 6.4 kcal/mol upon protonation at this position. While we considered the protonated form here for the sake of the swapped-ligands analogy as described in the Supporting Information (see also ref.[14]), it must be noted that protonation/deprotonation events have been shown to play a vital role in the catalytic cycle of Hmd.[33]

Interestingly, for two of the studied variants, [Fe]**1** and [Fe]**5**, O_2 addition is exothermic in both protonation states in contrast to the original native cluster ([Fe]). Especially in the deprotonated state, the altered ligand environment significantly favors O_2 binding to the central iron atom by 18 kcal/mol for [Fe]**1** and 20 kcal/mol for [Fe]**5** relative to the wild-type configuration. In the case of species [Fe]**5**, the pyridine ring of Hmd was replaced by a thiopyrane moiety to resemble the second coordinating sulfur atom of the dithiolate ligand in [FeFe] hydrogenase. This leads to two possible conformations of the hydroxy group in the 4-position to the sulfur atom of the thiopyrane ring. The isomer depicted in Figure 4 undergoes rearrangement upon structure optimization. This leads to the formation of a hydrogen bond between the H atom of the hydroxy group and the N atom of the CN^- ligand that is not present in the free form of the cluster and that obviously favors dioxygen binding. As a result, the alternate isomer [Fe]**5b** (not shown) coordinates O_2 with a reduced exothermicity of –4.4 kcal/mol (–1.1 kcal/mol for the protonated form). In the case of compounds [Fe]**2** and [Fe]**3**, which feature a thiabenzene ring in order to mimic a [FeFe] hydrogenase-like coordination sphere, O_2 binding is endothermic. The introduction of a CN^- ligand instead of CO even increases the endothermicity compared to that of the wild-type ligand arrangement. Here, in the O_2-free form, a proton transfer takes place from the hydroxy group *ortho* to the coordinating sulfur atom to the N atom of the CN^- ligand. Notably, the presence of CN^- instead of CO renders the energetics of O_2 coordination more exothermic for the wild-type and for the thiopyrane-based compound. In fact, the most significant shift to exothermic O_2 binding energies is observed for the original configuration when CO is replaced by CN^-, identifying its pivotal importance for the energetics of O_2 binding to the central Fe atom ([Fe] → [Fe]**1**, [Fe]**4** → [Fe]**5**).

It must be noted that the different Hmd species considered here do not carry identical charges, because of the ligand exchanges. In order to account for charge artifacts, we compare the coordination energies for the isolated species with results obtained with the COSMO continuum solvation approach for two different values of the dielectric constant ($\varepsilon = 4.0$ and $\varepsilon = 80.0$), which should be capable of stabilizing excess charges that might change the coordination energetics. The choice of the dielectric constant has hardly any effects on the results, and coordination energies obtained for $\varepsilon = 4.0$ and $\varepsilon = 80.0$ are basically identical (they differ by 0.4 to at most 1.3 kcal/mol), and we therefore report only one set of results. It turns out that the energy of O_2 binding is not much affected by solvent screening. Moreover, the difference in exothermicity between [Fe] and [Fe]**1** is not changed (see Table 2). Interestingly, for the other Hmd species considered, the changes due to solvation are even smaller with the exception of the protonated form of compound [Fe]**2** (Table 2; of the two isomers only [Fe]**5b** was considered because of its higher relevance).

For [Fe] and [Fe]**1** we also studied the change in energy induced by the chosen density functional and observe that in the deprotonated form the TPSS functional gives almost identical results as the BP86 functional, whereas with the B3LYP (hybrid) functional coordination energies are shifted to more endothermic values. For example, for the $^3O_2$ coordination energy to $[Fe]^{\pm 0}$ (isolated deprotonated form) we found 9.6 kcal/mol with BP86, 9.0 kcal/mol with TPSS, 16.0 with B3LYP, and 18.2 kcal/mol with B3LYP*, while we have for $[Fe]1^-$ (isolated deprotonated form) –8.4 kcal/mol with BP86, –9.0 kcal/mol with TPSS, 5.5 with B3LYP, and 0.5 kcal/mol with B3LYP*. Hence, the trends are comparable in the deprotonated forms (and even more pronounced

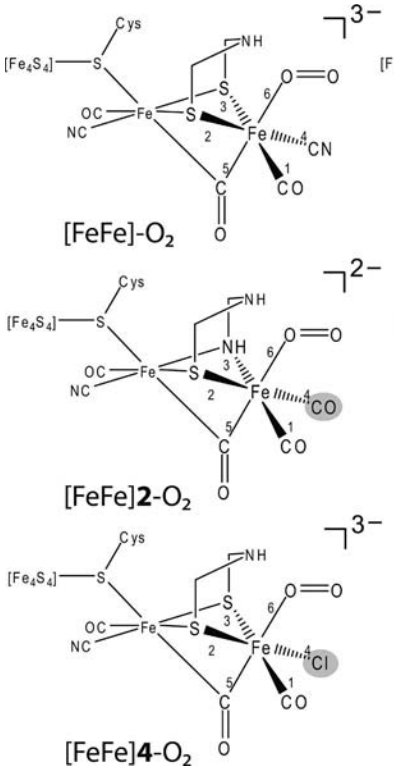

Figure 3. Lewis structures of dioxygen adducts of the H-cluster variants in different ligand environments. The given charges correspond to the active oxidized H_{ox}^{cat} form of the H-cluster. All complexes are considered in doublet spin state. Shaded ovals highlight substitutions at ligand position 4.

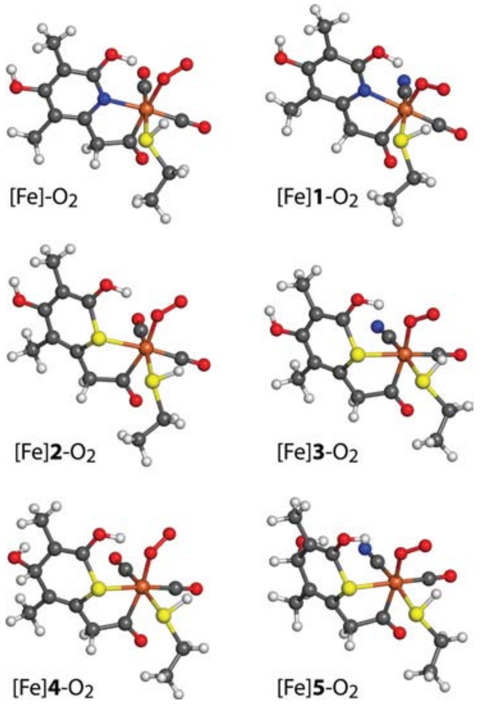

Figure 4. Structures of dioxygen adducts of Hmd variants optimized with BP86/TZVP. Charge and spin are the same as given in Figure 2. White: H, red: O, gray: C, blue: N, yellow: S, brown: Fe.

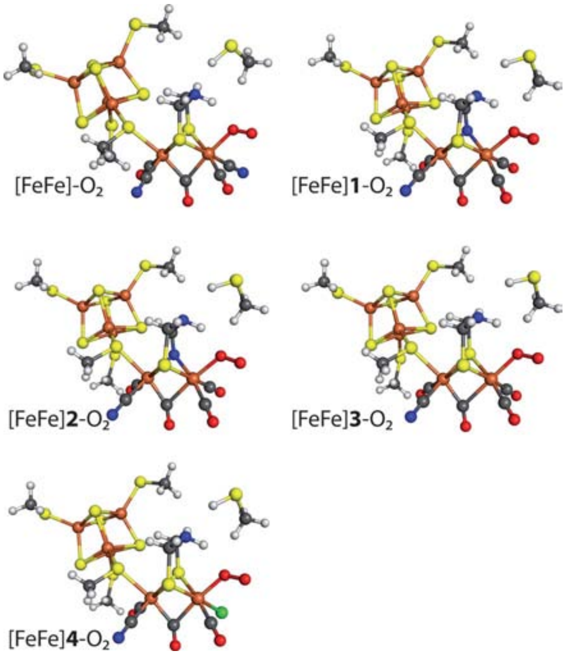

Figure 5. Structures of dioxygen adducts of H-cluster variants optimized with BP86/TZVP. Charge and spin are the same as given in Figure 3. White: H, red: O, gray: C, blue: N, yellow: S, brown: Fe, green: Cl.

in the protonated states), and we note the effect of the exact exchange admixture in the functional.

When we now compare with the O_2 coordination energies obtained for the studied H-cluster variants as given in

Table 1, we observe the reverse situation – swapping the first ligand sphere of the distal Fe_d atom into a Hmd-like coordination environment indeed reduces the exothermicity of O_2 binding. Importantly, this result is independent of the bridgehead group and of the O_2 binding mode. With an NH moiety in the bridgehead position, the reaction energy for O_2 coordination to the distal Fe_d drops from –16.9 kcal/mol for the wild-type configuration ([FeFe]) to –10.5 and –12.1 kcal/mol for the swapped compounds [FeFe]**1** and [FeFe]**2**. For the additional isomeric dioxygen adducts considered the effect of the changed ligand environment is significantly more pronounced and leads even to endothermic coordination energies (Table 1). However, this result might partly be due to changes in cluster geometry. For the two swapped Hmd-like H-cluster variants [FeFe]**1** and [FeFe]**2**, the Fe···Fe distance is decreased relative to that in the native cluster, and the bonding distance between the nitrogen-based ligand (ligand position 3) and the Fe_d atom is significantly smaller than the distance between the latter and the sulfur ligand in the original complex (see Table S2 in the Supporting Information). These geometrical changes seem to render the addition of O_2 in between the Fe_p and the Fe_d atom energetically more unfavorable, which is therefore maybe not a pure ligand field effect in contrast to the situation for the terminal dioxygen adducts.

In order to analyze the importance of the CN^- ligand that coordinates the Fe_d atom in isolation, we also consider here the single exchange by a CO moiety, as in the study of Hmd discussed above. Remarkably, also in this case we find a reduction in the exothermicity of O_2 coordination from wild-type levels of –16.9 to –10.7 kcal/mol for the active oxidized form. Therefore, it appears that substituting a single CN^- ligand at the catalytically active Fe_d atom alone reduces the exothermicity of O_2 coordination, which is in accordance with the reverse effect observed for Hmd as described above.

Again, the question rises whether the differences in exothermicity between the considered H-cluster derivatives are in fact mere charge effects due to a neglect of the protein environment. In order to account for this possibility, we again compare with results from COSMO calculations and observe that if we take the solvent into account implicitly, we cannot detect any significant deviations in the coordination energies (see Table 1, second numbers for the terminal oxygen adducts).

Compared to Hmd, the situation in [FeFe] hydrogenase is complicated due to the different redox states the H-cluster can adopt during its catalytic cycle. We therefore also analyze O_2 coordination to the reduced form of the H-cluster, H_{red}^{cat}, as summarized in Table 3 [note that we did not obtain converged structures for the isolated ("gas-phase") native cluster because of the highly negative charge of –4e]. The results were obtained by using the COSMO approach with an electric permittivity of $\varepsilon = 4.0$. Interestingly, the differences in exothermicity of O_2 addition to the distal Fe_d atom become more pronounced for the reduced forms. Furthermore, it is obvious that the differences in coordination energies between the native and the swapped clusters are no charge artifacts. Compound [FeFe]**1**, for example, binds O_2 with a coordination energy of only –2.6 kcal/mol in the reduced H_{red}^{cat} form, which has the same overall total charge of –3e as [FeFe] in the oxidized state that shows a coordination energy of –17.5 kcal/mol (BP86/COSMO). Note, however, that the active oxidized forms are in the doublet spin state, whereas the reduced clusters feature singlet spin multiplicity.

Table 3. BP86/TZVP reaction energies for the formation of O_2 adducts of the considered [FeFe] hydrogenase variants (H_{ox}^{cat} form), (NH in bridgehead position). Energies were obtained by employing the COSMO approach to account for solvent effects ($\varepsilon = 4.0$).

Educt	Terminal binding mode
$[FeFe]^{4-}$	–12.0
$[FeFe]\mathbf{1}^{3-}$	–2.6
$[FeFe]\mathbf{2}^{3-}$	–0.2
$[FeFe]\mathbf{3}^{3-}$	–1.3
$[FeFe]\mathbf{4}^{4-}$	–17.9

In order to understand the role of a strong and negatively charged π-acceptor, we also consider the replacement of CN^- by Cl^- at ligand position 4. Strikingly, even this substitution does not interfere with cluster integrity, both for the free and the dioxygen-bound form, and we obtain a reduced exothermic coordination energy of –12.5 kcal/mol, which is similar to that observed for the swapped variant [FeFe]**2**. However, as for the wild-type configuration with CN^- in ligand position 4, the chloride is bent significantly out of the octahedron's central tetragonal plane in the O_2-free form and is in hydrogen-bonding distance with the H atom of the bridgehead NH group (see Figure 6). This interaction could stabilize the free relative to the O_2-coordinated form and thereby render the coordination energy more endothermic. This becomes evident when we compare with the corresponding reaction energies for the variants that feature an O atom in the bridgehead position and for which no hydrogen-bonding interaction with ligand 4 is possible: here, the wild-type and the CN^-/Cl^--substituted complexes show al-

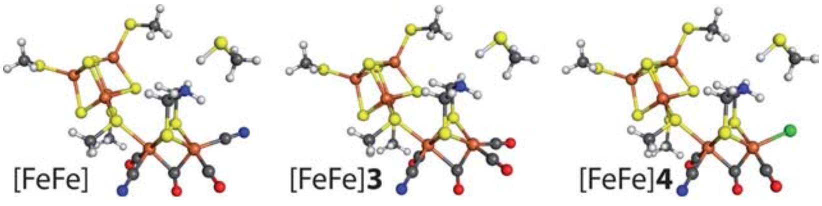

Figure 6. Structures of the dioxygen-free forms of the H-cluster variants [FeFe], [FeFe]**3**, and [FeFe]**4** optimized with BP86/TZVP. White: H, red: O, gray: C, blue: N, yellow: S, brown: Fe, green: Cl.

most the same O_2 coordination energy. Therefore, it seems likely that the decrease in exothermicity for the variants with NH in the bridgehead position is due to a strong hydrogen bond between the chloride and the H atom of the NH group.

The fact that a strong π-acceptor can be substituted by Cl^- without showing significant effects on cluster structure and O_2 coordination energy is a surprising result. However, the coordination energy gives no information about the overall stability of the cluster when CN^- is replaced by Cl^-. And indeed, by performing a hypothetical ligand exchange reaction of the type shown in Equation (3), we obtain a clearly endothermic reaction energy of 15.7 kcal/mol (18.1 kcal/mol with COSMO, $\varepsilon = 4.0$), which shows that the strong π-acceptor CN^- is significantly more tightly bound to the Fe_d atom than Cl^- and should presumably be important for the structural integrity of the active site under natural conditions.

$$[FeFe]^{3-} + Cl^- \rightarrow [FeFe]^{3-} + CN^- \qquad (3)$$

Given the energetics of O_2 coordination, the question can be asked whether the mode of O_2 coordination (i.e., its conversion to superoxide, for example) differs for the Hmd-based variants and the complexes that feature H-cluster type architecture. In terms of O–O bond lengths as given in Table S3 in the Supporting Information, it seems likely that for all considered cases the bound O_2 species in fact correspond to the superoxide form: all O–O bond lengths obtained lie within the range given by the experimental values for the superoxide ion in KO_2 (1.28 Å[43]) and for superoxide in the solid phase (1.33 Å[44]). However, the O–O bond length is obviously affected by the protonation state of the cysteine ligand in case of the Hmd-derived compounds, for which one can observe shortening upon deprotonation. Furthermore, replacing the CO ligand by CN^- elongates the O–O bond length, which suggests that it can play a role in activating the bound O_2 species. Taken together, the data suggest that the introduction of a single CO ligand instead of CN^- at the distal Fe_d atom disfavors the coordination of O_2 and its conversion to superoxide. Interestingly, we observe exactly the inverse effect for the Hmd active site, in which the substitution CO/CN^- shifts the energetics of O_2 binding and reduction from endothermic to exothermic.

5. Discussion

In order to understand the problem of dioxygen-induced inactivation of the H-cluster, it is of central interest to analyze by which factors its reactivity is affected. Here, we have to discriminate between the influence of the first coordination sphere of the catalytically active Fe_d atom and the impact of the surroundings, that is, the embedding of the H-cluster in its protein environment and the coupling to the electron-transfer chain. Interestingly, [FeFe] hydrogenases from different genetic backgrounds vary in their sensitivity against O_2, which suggests remote effects on the active site, such as changes in the redox potential induced by structural variations. In this work we have focused on the question of how the composition of the first coordination shell affects oxygen-induced inhibition of the H-cluster. This question is also important for the synthesis of biomimetic compounds that are not protected and functionally enhanced by virtue of a complex protein scaffold.

The presented data show that the difference in dioxygen sensitivity of Hmd and [FeFe] hydrogenase is paralleled by the energetics of O_2 coordination to the catalytically active iron centers. By applying our unifying structural and electronic concept of swapped ligand spheres,[14] which establishes a connection between both active sites, we are able to better understand the influence of ligand composition on dioxygen affinity.

Remarkably, we see a clear inversion in dioxygen affinity when we mutually exchange the ligand composition of the catalytically active Fe atoms of the active sites of [FeFe] and Hmd hydrogenase with one another. O_2 coordination to Hmd becomes favorable in a coordination sphere that resembles the ligation of the Fe_d atom in the H-cluster, whereas at the same time O_2 binding to the Fe_d atom of [FeFe] hydrogenase is less exothermic in an Hmd-like ligand configuration. The ligand field of the first coordination sphere therefore seems to have a significant influence on the dioxygen affinity of the central iron atoms.

Importantly, the theoretical analysis of the ligand environment discussed here reveals that the mutual exchange of CN^- by CO has the strongest impact on dioxygen binding and suffices to render O_2 coordination exothermic for Hmd and less exothermic for [FeFe] hydrogenase. This raises the general question of the role of a cyanide moiety at ligand position 3 for the mechanistic properties of the cluster. Interestingly, we find that in all ligand configurations that do not feature a CN^- group at this position, the spin distribution on the $2[Fe]_H$ subsite is affected, and one can observe a redistribution from the distal Fe_d atom in the wild-type configuration toward the proximal Fe_p atom accompanied by a general reduction in local spin density (see Supporting Information for details). This observation holds only for the uncoordinated forms, because in all cases a spin excess corresponding to one electron can be found on the two oxygen atoms of the bound O_2 species. In contrast to the substitution of CN^- by CO, the spin localization on the distal Fe_d atom does not change when a σ-donor-like chloride is introduced at ligand position 4. Moreover, also the energetics of O_2 coordination is less affected by this choice. Because of the different nature of the σ-donor Cl^- and the π-acceptor CN^-, it appears that the spin localization at Fe_d and the strength of O_2 coordination are predominantly determined by the negative charge provided by these ligands. Of course, we do not account here for possible mechanistic implications of the ligand environment on hydrogen formation or oxidation. Clearly, the observed variations in spin distribution will have an impact in this context, and we will address this question in future work. As outlined by Bruschi et al.,[40] the substitution of CN^- by CO affects the localization of the frontier orbitals and the electron transfer between the $[Fe_4S_4]$ cubane and the $2[Fe]_H$ subcluster. CN^- guarantees that, in the active reduced form, the HOMO is localized on the 2[Fe]$_H$ subsite, which facilitates proton binding to the distal Fe$_d$ atom. Therefore, it seems likely that the changes in oxygen coordination energy introduced by the exchange CN$^-$/CO are accompanied by mechanistic changes for the normal catalytic activity of the cluster. The presence of two CO groups in the active site of Hmd can be explained by the differing mechanistic requirements of this enzyme that shows no changes in the redox state of the mononuclear Fe core. Rather, hydrogen splitting is facilitated here in a heterolytic fashion by the presence of the cofactor methenyl-H4MPT$^+$.[33]

6. Conclusion

To conclude, we should summarize the most important results presented in this work.

(1) Coordination of triplet dioxygen to the active site of Hmd is found to be endothermic, which can explain the insensitivity of Hmd against oxygen.[45]

(2) This raises the question as to why coordination of $^3O_2$ to the distal iron atom in the active site of [FeFe] hydrogenase is exothermic, which we studied by a stepwise change of the first-shell ligand sphere at the reactive iron center from that of Hmd to that of [FeFe] hydrogenase on the basis of the similarities of both ligand environments highlighted in a previous paper.[14]

(3) We find that mainly the ligand in position 4 is responsible for the endo- or exothermicity of $^3O_2$ coordination. Spin localization at the distal Fe$_d$ atom and O_2 binding are favored by the presence of a negatively charged ligand at ligand position 4.

(4) Finally, we find that coordination energies for triplet oxygen are not very large in absolute value, which explains the fact that one hydrogenase binds it exothermically, while another one does not bind it at all. Although we have studied these effects for modified ligands directly bound to the reactive iron atom, we may anticipate that, because of the small magnitude of the coordination energies, mutagenesis studies that change the amino acid sequence in the proximity of the active site could provide [FeFe] mutants for which oxygen coordination is endothermic, so that the first step of the decomposition of the [FeFe] active site is not feasible.

It remains to be investigated how the modified ligand environments affect the regular catalytic cycle of the enzyme. Considering the fact that Hmd and [FeFe] hydrogenases perform different catalytic reactions, it is particularly important to study in what direction the hydrogen production equilibrium is shifted when the first-shell ligands are exchanged stepwise. This requires the localization of the relevant transition states, which is work in progress in our laboratory.

Supporting Information (see footnote on the first page of this article): Additional information on computational methodology; principle of swapped active sites; influence of ligand composition on dioxygen sensitivity; effects of ligand composition on spin distribution.

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