Electric-field effects on the [FeFe]-hydrogenase active site†

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The effect of a homogeneous electric field—as exerted by the protein environment and by an electrode potential—on the reactivity of the active site of [FeFe] hydrogenases is unravelled by density functional theory calculations.

Hydrogenases have been applied in electrochemical processes to produce molecular dihydrogen for clean-energy technologies.1 Electric fields affect the electronic structure and thus the reactivity of the active site. It is known that [NiFe] hydrogenases adsorbed on electrodes experience dispersion of reaction rates.2 This can be attributed to different orientations at the electron-transfer interface, though other effects, like double-layer potentials, might also be important.2,3 In general, little is known about the details of the interaction of stable and reactive intermediates with external fields in such situations. Here, we systematically investigate to what extent such fields can modify the energetics of key reaction steps at the active site of [FeFe] hydrogenases, which is the most desirable candidate for dihydrogen production. We should note that studies in a similar spirit have been performed for cytochrome P450 and proton transfer in a DNA base pair.4

[FeFe] hydrogenases catalyze the reversible formation of H2 with a high turnover frequency.5 The active site, the H-cluster, consists of a [4Fe–4S] cubane linked via a cysteine bridge to the so called [2Fe] subcluster. The latter comprises two iron atoms, one proximal (FeP) and one distal (Fed) to the cubane, and an azadithiolate bridging ligand.6 H2 formation at the H-cluster comprises proton/electron transfer steps and proceeds via a H– species terminally bound to FeP.7,8 A crucial decomposition reaction, which one seeks to avoid, is the O2-induced inhibition that initiates the degradation of the enzyme starting with O2 coordination to Fed.9

Chemical processes at active sites of such metalloproteins are usually well described by a quantum mechanical (QM) model that considers only the active site and some important amino acid residues.10 While the electric field of a surrounding protein may be approximated by a polarizable dielectric continuum model in a sufficiently large QM model, the strength of electric fields exerted on proteins adsorbed on polarized electrodes is difficult to assess.11

In order to systematically screen electric-field effects on our 96-atom QM model (Fig. 1, left), we first need to understand the magnitude and direction of the field exerted by the protein itself on the active site. We extract this information from the electrostatic potential that we obtain by numerical solution of the Poisson–Boltzmann equation for the crystal structure of [FeFe] hydrogenase from Clostridium pasteurianum (pdb code: 3C8Y) and from Desulfovibrio desulfuricans (pdb code: 1HFE) (detailed information can be found in the ESI†).12 The field within a protein is then obtained as the derivative of the electrostatic potential at the position of interest; in our case at the position of the Fed atom. The resulting local field vectors at FeP, EProt (Fed), are shown in Fig. 1 (red vectors) and were found to have a length of 0.0038 for C. pasteurianum and of 0.0026 for D. desulfuricans, both measured in Hartree atomic units (a.u.), in which the elementary charge and 4πε0 assume a value of one. Remarkably, these two field strengths are very
The change in partial charges on individual atoms induced by the electric field is small and discussed in the ESI. A clear trend is only revealed when the partial charges of the [2Fe]H subcluster and the cubane (including sulphur atoms of coordinating cysteines) are separately added up. For all intermediates in the direction of \( E_{\text{prot}}^0 \) induces a shift of electron density from the cubane to the [2Fe]H subcluster. The negative charge on the cubane decreases by +0.09e to +0.17e for different intermediates (and the strongest field \( E_{\text{prot}}^0 \)). The positive charge on the [2Fe]H subcluster increases by −0.05e to −0.13e, accordingly. The decrease in negative charge on the cubane is always smaller than the increase in negative charge on the [2Fe]H subcluster because the charge is shifted to the amino acid environment of the [2Fe]H subcluster. \( E_{\text{Fe-Fe}} \) induces a transfer of negative charge from the cubane to the [2Fe]H subcluster. Inversion of the field direction leads to the expected inversion of the charge shifts. Hence, the protein field clearly polarizes the active site towards the [2Fe]H subcluster. The inverse field has a less pronounced effect. The polarization could be enhanced by a field in the \( E_{\text{Fe-Fe}} \) direction (or lowered by \(- E_{\text{Fe-Fe}}\)). The cubane serves as a charge reservoir and \( E_{\text{Fe-Fe}} \) and \( E_{\text{prot}}^0 \) increase charge donation from the cubane and to the [2Fe]H subcluster.

The effect of the field on reaction energies of reaction steps in Fig. 2 of the catalytic cycle\(^\dagger\) are summarized in Table 1 (see ESI for data of inverted fields). In reaction [FeFe]H\(^+\) → TSI → [FeFe] − H\(^+\) (rows 1–3 in Table 1) a proton is transferred from the bridgehead amine group to Fe\(_6\) to form the terminal hydride species. This reaction becomes less exothermic with increasing field in \( \vec{E} \) strength. For \( \vec{E}_{\text{prot}}^0 \) it is +10.7 kcal mol\(^{-1}\) less exothermic than in the field-free case. The reaction energy changes linearly with the field. For each increase in field strength the reaction becomes +1.6 kcal mol\(^{-1}\) less exothermic. By contrast, \( \vec{E}_{\text{Fe-Fe}} \) leads to a −4.9 kcal mol\(^{-1}\) more exothermic reaction. For a less exothermic reaction the barrier is higher, while it is lower for a more exothermic reaction, but the change in barrier height is smaller than the change in reaction energy. The effect of \( E_{\text{prot}}^0 \), \( E_{\text{prot}}^\dagger \), and \( E_{\text{prot}}^0 \) on the energy profile of the reaction [FeFe]H\(^+\) → TSI → [FeFe] − H\(^+\) (terminal hydride formation) is shown in Fig. 3. For the H\(_4\) formation reaction, [FeFe]H\(^+\) − H\(^-\) → [FeFe] − H\(_3\), the reaction is up to +4.3 kcal mol\(^{-1}\) less exothermic with increasing field in \( E_{\text{prot}}^0 \) direction. This decrease

### Table 1

| Intermediate | No field \( \vec{E}_{\text{prot}} \) | \( \vec{E}_{\text{prot}}^0 \) | \( \vec{E}_{\text{prot}}^\dagger \) | \( \vec{E}_{\text{prot}}^0 \) | \( \vec{E}_{\text{Fe-Fe}} \) | \( \vec{E}_{\text{Fe-Fe}} \) |
|-------------|----------------|----------------|----------------|----------------|----------------|----------------|
| H\(_4\) formation | [FeFe]H\(^+\) → TSI → [FeFe] − H\(^+\) | −20.7 | −19.5 | −17.9 | −16.3 | −14.8 | −13.2 | −11.6 | −10.0 | −25.6 | −17.5 |
| [FeFe]H\(^+\) → TSI | 4.0 | 4.0 | 4.4 | 4.7 | 5.1 | 5.5 | 5.8 | 6.2 | 3.4 | 4.2 |
| [FeFe]H\(^+\) − H\(_2\) → [FeFe] − H\(_2\) | −6.4 | −5.8 | −5.2 | −4.6 | −4.0 | −3.4 | −2.8 | −2.1 | −0.9 | −2.4 |
| O\(_2\) additions | [FeFe]\(_{\text{ox}}\) → [FeFe]\(_{\text{ox}}\) − O\(_2\) | −23.6 | −23.4 | −22.9 | −22.2 | −21.6 | −21.0 | −20.3 | −19.6 | −19.7 | −27.2 |
| [FeFe]\(_{\text{ox}}\) → [FeFe]\(_{\text{red}}\) − O\(_2\) | −17.2 | −16.8 | −16.3 | −15.8 | −15.3 | −14.8 | −14.3 | −13.8 | −15.6 | −20.3 |
| Reduction | [FeFe]\(_{\text{ox}}\) → [FeFe]\(_{\text{red}}\) | 77.9 | 53.5 | 22.4 | −8.6 | −39.6 | −70.7 | −101.7 | −132.8 | 73.7 | 83.5 |
| [FeFe]\(_{\text{ox}}\) → [FeFe]\(_{\text{red}}\) | 70.0 | 45.5 | 14.9 | −15.8 | −46.4 | −77.0 | −107.6 | −138.2 | 62.8 | 78.4 |
in exothermicity is linear as well. Importantly, both fields, $\tilde{E}_{\text{Fe-Fe}}$ and $-\tilde{E}_{\text{Fe-Fe}}$ lead to a decreased exothermicity (+5.5 and +4.0 kcal mol$^{-1}$, resp.) for H$_2$ formation. Hence, $E^\mu_{\text{prot}}$ disfavors both proton transfer reactions, while $\tilde{E}_{\text{Fe-Fe}}$ favors hydride transfer but disfavors H$_2$ formation.

The reduction of the H$_2$-coordinated species and the species with a free coordination site in the $\mu$-bridging position are, of course, most affected by the electric field (last two rows in Table 1). Surprisingly, $E_{\text{Fe-Fe}}$ has only a small effect on the energies. Moreover, with $E^\mu_{\text{prot}}$ the O$_2$ coordination energies become +4.0 and +3.4 kcal mol$^{-1}$ less exothermic for the oxidized and reduced oxidation states, respectively, although the coordination reaction remains strongly exothermic.

To conclude, the polarization of the H-cluster induced by $E_{\text{Fe-Fe}}$ is stronger than that induced by $E^\mu_{\text{prot}}$. Inversion of both fields leads to inversion of differential polarization but with smaller magnitude. This is due to the excess of charge on the cubane in most intermediates. $E^\mu_{\text{prot}}$ leads to a reduced exothermicity of hydride and H$_2$ formation which could be beneficial for the enzyme to work close to the thermodynamic equilibrium. Our results explicitly show that no crucial field-induced modulations of barrier heights and reaction energies are observed. The reversibility of the whole H$_2$ formation cycle is not affected. Still, we might speculate that the [FeFe] hydrogenase from $C$. pasteurianum should feature an activation pattern that favors H$_2$ oxidation and disfavors H$_2$ formation compared to the one from $D$. desulfuricans because the former exerts a stronger field at the ligand-binding site. The measured activity data appear to indicate such a trend for H$_2$ evolution.$^{14}$

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