Supporting Information

Efficient and Selective Electrochemically-Driven Enzyme-Catalyzed Reduction of Carbon Dioxide to Formate using Formate Dehydrogenase and an Artificial Cofactor

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Supporting Information 1

Thermodynamics of FDH catalyzed CO₂ reduction using MV radical cation

Redox reaction of CO₂ reduction to formate by MV radical cation gives a free energy of 15.4 kJ/mol (3.68 kcal/mol) at pH 7 (Eq.4).

\[
\begin{align*}
\text{CO}_2 + 2\text{H}^+ + 2e^- & \rightarrow \text{HCOOH} & E^o &= -0.19 \text{V vs NHE} \quad (1) \\
\text{MV}^{2+} + e^- & \rightarrow \text{MV}^{+} & E^o &= -0.44 \text{V vs NHE} \quad (2) \\
\text{CO}_2 + \text{H}_2\text{O} + 2e^- & \rightarrow \text{HCOO}^- + \text{OH}^- & E^o_{[\text{pH}=7]} &= -0.52 \text{V vs NHE} \quad (3)
\end{align*}
\]

\[
(3)-(2) \quad \Delta E = E^o(3) - E^o(2) = -0.08 \text{ V}
\]

\[
\Delta G^o = -nF\Delta E \quad \text{where} \ n = \text{number of electrons and} \ F = \text{Faraday constant}
\]

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} + 2\text{MV}^{2+} & \rightleftharpoons 2\text{MV}^{3+} + \text{HCOO}^- + \text{OH}^- & \Delta G^o &= 15.4 \text{ KJ/mol (3.68 kcal/mol)} \quad (4)
\end{align*}
\]

Equilibrium constant of CO₂ reduction using MV radical cation is \(2x10^{-3}\).

\[
\Delta G = \Delta G^o + RT \ln K_{\text{MV}}
\]

At the Equilibrium, \(\Delta G^o = -RT \ln K_{\text{MV}}\)

\[
K_{\text{MV}} = \frac{[\text{HCOO}^-][\text{MV}^{2+}]^2}{[\text{CO}_2][\text{H}^+][\text{MV}^{3+}]^2} \quad \text{at 298K,} \ K = e^{-\frac{\Delta G^o}{RT}} = 2.0 \times 10^{-3}
\]
Thermodynamics of FDH catalyzed CO$_2$ reduction using NADH

Redox reaction of CO$_2$ reduction to formate by NADH gives a free energy of 38.6 KJ/mol (9.23 kcal/mol) at pH 7 (Eq.6).

\[
\text{NAD}^+ + 2e^- + H^+ \rightarrow \text{NADH} \quad E^o_{\text{[pH=7]}} = -0.32 \text{ V vs NHE} \tag{5}
\]

\[
\Delta E = E^o(3) - E^o(5) = -0.20 \text{ V}
\]

\[
\Delta G^o = -nF\Delta E
\]

\[
\text{CO}_2 + \text{NADH} \rightleftharpoons \text{NAD}^+ + \text{HCOO}^- \quad \Delta G^o = 38.6 \text{ KJ/mol (9.23 kcal/mol)} \tag{6}
\]

Equilibrium constant of CO$_2$ reduction using the natural co-factor, NADH is 1.7 x10$^{-7}$.

\[
\Delta G = \Delta G^o + RT \ln K_{\text{NAD}}
\]

At the Equilibrium, $\Delta G^o = -RT \ln K_{\text{NAD}}$

\[
K_{\text{NAD}} = \frac{[\text{HCOO}^-][\text{NAD}^+]}{[\text{CO}_2][\text{NADH}]} \quad \text{at 298K, } K = e^{-\frac{\Delta G^o}{RT}} = 1.7 \times 10^{-7}
\]

Ratio of $K_{\text{MV}}/K_{\text{NAD}}=10,000$

Equilibrium potential at our experimental conditions can be represented as per Eq.7$^1$.

\[
E^o' = E^o - \frac{RT}{nF} \ln \left( \frac{[\text{HCOO}^-][\text{H}^+]^2}{[\text{HCO}_3^-]} \right)
\]

\[
E^o' = 0.078 - 0.059\text{pH} - 0.030 \log \left( \frac{[\text{HCOO}^-]}{[\text{HCO}_3^-]} \right) \tag{7}
\]

\[
E^o'_{\text{[pH=6.6,0.1 M HCO}_3^-0.004 \text{ M HCOO}^-]} = E^o - 0.059(\text{pH}) - 0.030 \log \left( \frac{[\text{HCOO}^-]}{[\text{HCO}_3^-]} \right)
\]

\[
E^o' = 0.078 - 0.389 + 0.042 = -0.42 \text{ V vs NHE}
\]
Supporting Information 2

Figure S1: Detection of MV di-cation and reduced form during bulk electrolysis

MV in its reduced form was synthesized in a three electrode system using Toray paper working electrode, platinum counter electrode and Ag/AgCl reference electrode at -0.45 V vs NHE. The concentration of MV in its redox states was followed by scanning voltammetry (Figure S1) using a carbon fiber microelectrode (Basi, MF-2007).²
Supporting Information 3

Figure S2: UV/Vis detection of MV$^{**}$ generation during formate oxidation with MV$^{2+}$ using FDH

FDH (3.6 μM) and sodium formate (0.5 M) were added into the phosphate buffer (0.5 M, pH=7, 3 mL). Argon gas was bubbled before adding methyl viologen (20 mM) to the reaction mixture. No changes in the absorption intensity at 606 nm (characteristic peak to MV$^{**}$)$^3$ were observed in 6 hrs (Figure S2).
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Figure S3: Crystal structure of cbFDH bound to NAD$^+$ and azide (PDB ID: 5DN9). The docking box used to dock MV$^{++}$ is shown in magenta.

Docking of methyl viologen, bicarbonate and hydronium ions to FDH

MV$^{++}$ was docked to the active site of cbFDH bound to NAD$^+$ and azide ion (PDB ID: 5DN9)$^4$ using single precision Glide$^5,6$. The receptor was prepared and minimized using the Protein Preparation Wizard of the Maestro interface (Schrodinger™ LLC.). The hydrogen atoms were added and protonation state of histidine residues were determined using ProtAssign$^7$. The center of mass of the bound NAD$^+$ was chosen as the center of the docking box, as shown in Figure S3. The vdW radii of both the receptor and ligand atoms were scaled by 0.8. The partial charges of the ligands MV$^{++}$, H$_3$O$^+$ and HCO$_3^-$ were calculated using quantum mechanical geometry optimization using the Hartree-Fock theory with 6-31G** basis set and a PBF water solvation model in Jaguar$^8$. The docked poses of MV$^{++}$ were clustered by RMSD and the best pose was selected based on both low glide score and overlap with the bound NAD$^+$ in the crystal structure. Next, we replaced the azide ion in the crystal structure with the bicarbonate ion and docked H$_3$O$^+$ to the active site with MV$^{++}$ and HCO$_3^-$ present, using the same docking method as MV$^{++}$. For docking H$_3$O$^+$, the center of the docking box was placed on the bicarbonate ion. The best pose for H$_3$O$^+$ was selected based on proximity to both bicarbonate and MV$^{++}$. Using the docked structure of FDH bound to the first MV$^{++}$ molecule, the second MV$^{++}$ was docked using a similar protocol as given above. The structure of the product bound FDH was obtained from the reactant bound FDH by editing the reactant molecules in the Builder.
module of Maestro. As with the reactants, the partial charges of the product molecules, MV\(^{2+}\), formate and OH\(^-\) ions were also calculated using QM geometry optimization in Jaguar.

**Molecular dynamics simulations of methyl viologen and NADH bound FDH**

The structures of MV\(^{**}\) and MV\(^{2+}\) bound FDH were obtained from the docking calculations described in the previous section. The structures of NADH and NAD\(^+\) bound FDH were obtained from the crystal structure of NAD\(^+\) bound FDH (PDB ID: 5DN9). Both formate and bicarbonate ions were placed at the location of the azide molecule in the crystal structure. The protein structures were solvated in explicit water, and sodium and chloride ions were added to neutralize the system charges. The systems were parameterized using the AMBER FF14SB force field for protein\(^9\), GAFF2 for ligands\(^\text{10}\) and the TIP3P water model for solvent\(^\text{11}\). The simulations were performed using the AMBER16 software package on a GPU cluster comprising NVIDIA P100 GPUs. The systems were initially minimized for 2000 steps using conjugate gradient minimization, followed by gradual heating to 310K over 50 ns in the NVT ensemble. Then, a 20 ns equilibration was performed in the NPT ensemble at 310K and a pressure of 1 atm. During these steps, the protein and ligand heavy atoms were restrained with a force constant of 500 kcal/mol. Next, the system was relaxed while slowly reducing the restraining force over 50 ns (NPT ensemble, 310K, 1 atm). Finally, a 50 ns equilibration was performed on each system without any restraints, before initiating the production simulations. Due to the instability of the OH\(^-\) and formate ions in MV\(^{2+}\) bound FDH (they leave the protein cavity within 6 and 50ns of starting unrestrained MD), we restrained these ions inside the binding cavity by imposing distance restraints with R258 and MV\(^{2+}\) using a flat bottom potential. The oxygen atom of OH\(^-\) was restrained to be within 6.5Å of the sidechain of R258, and 5Å of the nitrogen of the pyridine ring of MV\(^{2+}\). The oxygen atom of formate was restrained to be within 7.5Å of the sidechain of R258 and 5Å of the nitrogen of the pyridine ring of MV\(^{2+}\). Likewise, in the NADH bound FDH simulations (bicarbonate ion left the protein cavity in unrestrained MD within 30-80 ns), the bicarbonate ion was restrained to be within 5 and 6.5Å of the R258 and H311 sidechains respectively, and 5Å of the nicotine ring of NADH. Below these cutoff distances, the ions did not experience any force and were free to move. The production runs consisted of 3 independent simulations of 50 ns each in the NPT ensemble, starting from randomly selected velocities from the Maxwell-Boltzmann distribution.

**Clustering of trajectories and calculation of binding free energy**

The protein ligand complex conformations obtained from the MD simulations were clustered by protein backbone and ligand heavy atom RMSD using the hierarchical clustering routine in CPPTRAJ\(^\text{12}\), with a maximum RMSD cutoff of 1.5Å per cluster. The binding energy values were calculated using the MMPBSA program, which is part of the AMBERTOOLS17 package.
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Figure S4: Steady-state reduction CO$_2$ reduction (40 mM MV$^{2+}$, 2.6 uM FDH) and Nafion® 117 membrane; volume of each chamber 7.5 mL).

Supporting Information 6

Rate constant (k) was calculated as rate per unit enzyme concentration.

\[ k = \frac{\text{Rate}}{n \cdot [ET]} \]

where

Rate=Detected formate concentration/ time

n= number of active sites per unit of enzyme (for FDH from Candida boidinii n=2)

[ET] = enzyme concentration

When rate is at the maximum value for a given enzyme concentration, \( k=k_{\text{cat}} \).
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