

Reversible Interconversion of CO₂ and Formate by a Molybdenum-Containing Formate Dehydrogenase

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S Supporting Information

ABSTRACT: CO₂ and formate are rapidly, selectively, and efficiently interconverted by tungsten-containing formate dehydrogenases that surpass current synthetic catalysts. However, their mechanism of catalysis is unknown, and no tractable system is available for study. Here, we describe the catalytic properties of the molybdenum-containing formate dehydrogenase H from the model organism *Escherichia coli* (*EcFDH-H*). We use protein film voltammetry to demonstrate that *EcFDH-H* is a highly active, reversible electrocatalyst. In each voltammogram a single point of zero net current denotes the CO₂ reduction potential that varies with pH according to the Nernst equation. By quantifying formate production we show that electrocatalytic CO₂ reduction is specific. Our results reveal the capabilities of a Mo-containing catalyst for reversible CO₂ reduction and establish *EcFDH-H* as an attractive model system for mechanistic investigations and a template for the development of synthetic catalysts.

The efficient reduction of carbon dioxide (CO₂) to generate reduced carbon compounds for use as fuels and chemical feedstocks is an essential requirement for a carbon-based sustainable energy economy.¹ The electrochemical reduction of CO₂, powered by carbon-neutral electricity, would produce liquid fuels that are easier to store and transport than hydrogen, but only limited progress has been made in developing synthetic catalysts to overcome the kinetic and thermodynamic challenges of CO₂ activation. Catalysts developed so far are inefficient and expensive, due to their requirement for high overpotentials or their reliance on noble metals.^{2–9}

The rapid, reversible, and specific electrochemical reduction of CO₂ to formate by a tungsten-containing formate dehydrogenase from the anaerobic bacterium *Syntrophobacter fumaroxidans* (*SfFDH1*) provided the paradigm case for a formate/CO₂ catalyst.¹⁰ *SfFDH1* catalyzes the rapid interconversion of CO₂ and formate at the reduction potential for the reaction, establishing it as a thermodynamically reversible catalyst.¹¹ Therefore, the catalytic mechanism of CO₂ reduction by the W-center in *SfFDH1* is a valuable source of information to aid the design of improved synthetic catalysts. However, *SfFDH1* itself is intractable for mechanistic studies: cell cultures of *S. fumaroxidans* take several months to achieve low cell densities that provide only minuscule amounts of

enzyme; no genetic manipulation is possible; and the enzyme contains an extensive cohort of iron–sulfur (FeS) centers to transfer electrons to and from the active site, making overexpression strategies untenable.^{10,12,13} Therefore, a more versatile and robust experimental system is required.

SfFDH1 is a member of the large class of prokaryotic formate dehydrogenases that contain either Mo- or W-cofactors; they also contain a second, independent active site where quinone, protons, or NAD(P)⁺ react,^{14,15} which may be replaced functionally by an electrode to produce an electrocatalyst for CO₂/formate interconversion. The W-containing active site is known to be thermodynamically reversible¹⁰ but is found exclusively in anaerobic bacteria such as *Desulfovibrio*¹⁶ and *Eubacterium*¹⁷ species. Conversely, the thermodynamic reversibility of the more common molybdenum-containing active site remains to be established. Several indications that CO₂ reduction is possible have been reported: a multisubunit Mo-containing FDH from *Desulfovibrio vulgaris* Hildenborough has been reported to reduce CO₂ slowly in solution,¹⁸ and production of formate from CO₂ and H₂ was observed in early whole-cell experiments with *Escherichia coli*¹⁹ (suggesting the formate hydrogenlyase can operate in reverse), and from a putative Mo-containing FDH in the multisubunit H₂-dependent CO₂ reductase of *Acetobacterium woodii*.²⁰ Here, we define the catalytic properties of the structurally defined Mo-containing formate dehydrogenase H, a component of the formate hydrogenlyase complex in the model organism *E. coli* (*EcFDH-H*).²¹

EcFDH-H contains a Mo coordinated by a selenocysteine (SeCys) residue and two molybdopterin guanine dinucleotides (MGDs), and just one [4Fe-4S] cluster for electron transfer to and from the active site (Figure 1).²¹ It was produced by overexpression in *E. coli* under anaerobic growth conditions^{22,23} and purified by adapting a previously reported method²⁴ (see Supporting Information (SI) for details). All purification and experimental procedures were performed under strictly anaerobic conditions.

Figure 2 shows protein film voltammograms recorded at different pH values with *EcFDH-H* adsorbed on the surface of a graphite-epoxy rotating disk working electrode (geometric surface area 0.07 cm²; see SI for details). *EcFDH-H* catalyzes the reversible interconversion of CO₂ and formate with electrocatalytic characteristics similar to those observed previously for *SfFDH1*.¹⁰ Each set of voltammograms slices

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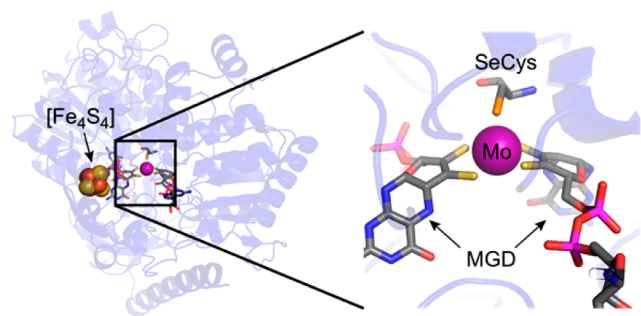


Figure 1. Structure of *EcFDH-H* showing the active site consisting of Mo coordinated by a SeCys and two MGDs and the [4Fe-4S] cluster that transports electrons to and from the active site. Figure generated with PyMOL from 1AA6 pdb.²¹

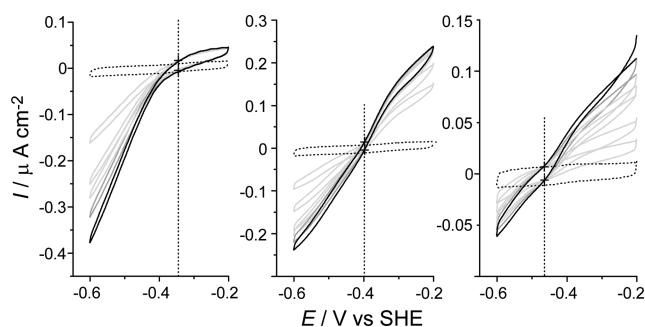


Figure 2. Cyclic voltammograms showing reversible CO_2 reduction and formate oxidation by *EcFDH-H* adsorbed on a graphite-epoxy electrode, pH 6.0 (left), 6.8 (middle), and 8.0 (right). The points of intersection (marked with crosses) define the reduction potentials for the CO_2 /formate interconversion (vertical lines). First voltammetric cycles are shown in black and subsequent cycles (2–4, 10, and 20) in gray. Voltammograms recorded in the absence of substrates are shown as dashed traces. Conditions: 10 mM CO_2 ,²⁵ 10 mM formate, 25 mM of each of four pH buffers (acetate, MES, HEPES, and TAPS), voltammetric scan rate 25 mV s^{-1} , electrode rotation rate 2000 rpm, 23°C .

cleanly through unique zero-current points, traced out as points of intersection as the current decays during successive scans. The zero-current points denote the thermodynamic reduction potential for the CO_2 /formate interconversion (net formate oxidation occurs at more positive potentials and net CO_2 reduction at more negative potentials), demonstrating that the electrocatalytic reaction is thermodynamically reversible.¹¹ Both the oxidation and reduction currents increase rapidly as the overpotential is increased but do not reach potential-independent limiting currents within the accessible potential range. This behavior is typical of highly catalytically active enzymes, for which the electrocatalytic rate is limited by interfacial electron transfer.¹¹

Figure 3 shows how the measured CO_2 reduction potentials vary with pH, and that they are consistent with potentials calculated using the Nernst equation and known pK values.^{10,26} They also match values measured previously using *SfFDH1*,¹⁰ with a small decrease in the predicted value of $E^{0'}$ attributable to the lower temperature used here. The data in Figure 3 are key evidence in establishing Mo-containing *EcFDH-H* as a catalyst specific for CO_2 /formate interconversion as well as a demonstration that catalysis remains reversible over a wide range of conditions.

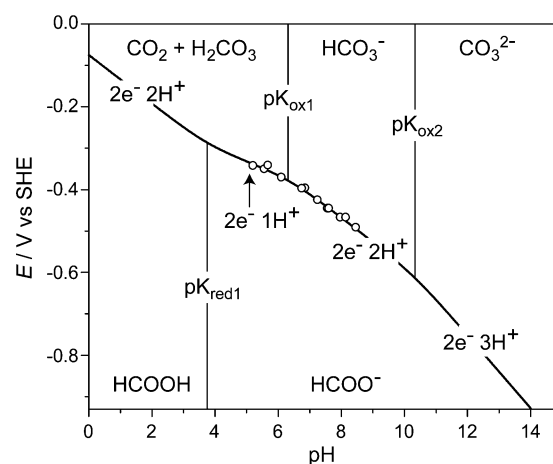


Figure 3. Reduction potentials for the CO_2 /formate interconversion measured using *EcFDH-H*, superimposed on a Pourbaix diagram to show the most stable species present under each condition. The reduction potentials (measured as illustrated in Figure 2) were recorded in 10 mM formate and 10 mM CO_2 ,²⁵ at 23°C and fitted to the Nernst equation (solid line)¹⁰ using $\text{pK}_{\text{red}1} = 3.75$, $\text{pK}_{\text{ox}1} = 6.39$, $\text{pK}_{\text{ox}2} = 10.32$,^{10,26} and $E^{0'} = -0.075 \text{ V}$.

To confirm the selectivity of catalysis, bulk CO_2 reduction was carried out using a graphite-epoxy “pot” working electrode (geometric surface area $\sim 5.3 \text{ cm}^2$) with *EcFDH-H* adsorbed to the surface. The current was recorded during electrolysis periods of 1–2 h at -0.5 and -0.6 V vs SHE ($\sim 110 \text{ mV}$ and 210 mV overpotential, respectively), and the total charge passed calculated by integration of the current over time (see Figure S2). Following electrolysis, analysis by ion chromatography revealed formate as the single observable product, formed with a Faradaic efficiency of $101.7 \pm 2.0\%$ (standard error measurement, $n = 3$). These data confirm formate as the quantitative product of the electrocatalytic reduction of CO_2 to formate by *EcFDH-H*.

Despite the Mo-containing *EcFDH-H* and the W-containing *SfFDH1* being similarly capable of the electrocatalytic interconversion of CO_2 and formate, their abilities to catalyze in solution-based assays are strikingly different. Standard FDH solution assays utilize either benzyl viologen (BV^{2+}) or methyl viologen (MV^{2+}) as the redox partner and monitor either formation (reduction of MV^{2+}) or consumption (oxidation of MV^{2+}) of the blue radical-cation reduced viologen ($\text{MV}^{\cdot+}$), coupled to formate oxidation or CO_2 reduction, respectively.^{12,27} Table 1 compares solution assay and electrochemical data from *SfFDH1* and *EcFDH-H*.

Both *EcFDH-H* and *SfFDH1* catalyze the rapid oxidation of formate coupled to the reduction of BV^{2+} , an electron acceptor with a reduction potential more positive than that of CO_2 (-0.36 V vs SHE).²⁸ For this reason, BV^{2+} is unsuitable for CO_2 reduction assays. Only *SfFDH1* is capable of rapid formate oxidation coupled to the reduction of MV^{2+} , an electron acceptor with a lower reduction potential (-0.45 V vs SHE)²⁸ that is comparable to that of CO_2 . Furthermore, solution measurements of CO_2 reduction using $\text{MV}^{\cdot+}$ revealed rapid formate production only by *SfFDH1*. Assays with *EcFDH-H* yielded turnover numbers for CO_2 reduction below 1 s^{-1} , more than 2 orders of magnitude slower than the rate of BV^{2+} -linked formate oxidation, or of the rates of both reactions catalyzed by *SfFDH1*. This result is likely the reason why CO_2 reduction by this Mo-containing FDH has not been observed previ-

Table 1. Comparison of Catalysis by S_fFDH1 and EcFDH-H in Solution Assays and Electrochemically (pH 7.5 ± 0.1, 23 °C)

reaction	W-S _f FDH1	Mo-EcFDH-H
formate + MV ²⁺	1500 s ^{-1a}	4 s ⁻¹
formate + BV ²⁺	est. 1100 s ^{-1b}	160 s ⁻¹
CO ₂ + MV ⁺	500 s ^{-1a}	<1 s ⁻¹
formate + electrode ^c	160 μA cm ^{-2a}	180 μA cm ⁻²
CO ₂ + electrode ^c	5 μA cm ^{-2a}	80 μA cm ⁻²

^aTaken from ref 10. ^bValue estimated from published values,¹² supported by preliminary in-house data. ^c250 mV overpotential for S_fFDH1, 150 mV for EcFDH-H. Solution assays contained 1 mM BV²⁺ or MV²⁺ for formate oxidation or 0.1 mM MV⁺ for CO₂ reduction. All experiments used 10 mM formate or 10 mM CO₂.²⁵

ously,^{24,27,29} leading to the general concept that the Mo-containing active site does not catalyze in a thermodynamically reversible manner.

Electrochemically, both enzymes catalyze in both directions with significant rates. In both cases formate oxidation increases, relative to CO₂ reduction, as the pH is increased, but at pH 7.5 EcFDH-H is biased more strongly toward CO₂ reduction than S_fFDH1 (Table 1), suggesting that the “operating potential”¹¹ of EcFDH-H is more negative than that of S_fFDH1. Although this interesting suggestion is contrary to expectations that the W-center should operate at a lower potential than the Mo-center, intramolecular electron transfer to and from the active site may also influence the catalytic bias.¹¹

The reason why EcFDH-H, despite being such a good electrochemical catalyst, is unable to catalyze CO₂ reduction by MV⁺ or formate oxidation by MV²⁺ is intriguing. We suggest two possibilities. First, it may be purified in an inactive state that cannot be recovered easily in solution-based assays. Attempts to use different conditions and pretreatments to reactivate the enzyme have failed to substantiate this suggestion, but catalytic lag phases observed in formate oxidation assays by S_fFDH1 (which can be avoided by pretreatment with MV⁺) suggest that inactive states of FDH enzymes can be formed. Second, the activity of EcFDH-H may be dominated by the single [4Fe-4S] cluster that transfers electrons to and from the active site. During formate oxidation the Mo-center readily reduces the cluster, but the cluster (having, we expect, a more positive reduction potential) is able to pass its electron efficiently only to BV²⁺, not MV²⁺. Similarly, for CO₂ reduction, MV⁺ readily reduces the cluster, but the electron tends to remain on the higher-potential cluster (blocking further electron transfers from MV⁺), rather than move to the active site. In contrast, on the electrode surface the abundance of electrons with sufficient driving force overcomes the FeS barrier to CO₂ reduction by backfilling the oxidized cluster immediately, when the electron moves otherwise transiently to the active site (and similarly, for formate oxidation it takes the electron from the cluster at the active site potential). In contrast to EcFDH-H, S_fFDH1 contains around 10 FeS clusters¹⁰ to buffer electron supply and demand. Our results highlight the problems of relying on inefficient and slow redox mediators to report on catalysis by a rapidly catalyzing, buried active site.

The reduction of CO₂ to liquid fuel products is currently of much interest, and there is a strong requirement for catalysts that operate efficiently, selectively and under mild conditions. Some ruthenium, iron, manganese, and copper based catalysts

are able to reduce CO₂ electrochemically, but large overpotentials are typically required and their efficiency is low.^{2–9} In contrast, formate dehydrogenases catalyze the two electron reduction of CO₂ directly to energy-rich formic acid with high selectivity, under mild conditions, with little overpotential requirement, and elucidation of their catalytic mechanism may inform the development of improved synthetic catalysts. Tungsten-containing S_fFDH1 from *S. fumaroxidans* set an important paradigm but is intractable for in-depth studies. Here we have demonstrated that molybdenum-containing EcFDH-H from *E. coli* is also capable of reversible, specific, and efficient CO₂ reduction, so the Mo-center is capable of reversible CO₂ reduction and provides a new blueprint for synthetic catalyst design. Based on its simplicity and relative ease of production and manipulation, we establish EcFDH-H as a new model system of choice for mechanistic investigations of enzymatic CO₂ reduction.

■ ASSOCIATED CONTENT

📄 Supporting Information

Experimental methods for protein preparation, solution assays, and electrocatalysis experiments, and comparison with the kinetic data of Axley and Grahame.²⁷ This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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