Reversible and selective interconversion of hydrogen and carbon dioxide into formate by a semi-artificial formate hydrogenlyase mimic

Katarzyna P. Sokol,[1,‡] William E. Robinson,[1,‡] Ana R. Oliveira,[2] Sonia Zacarias,[2] Chong-Yong Lee,[1] Christopher Madden,[1] Arnau Bassegoda,[3] Judy Hirst,[3] Inês A. C. Pereira,[2] Erwin Reisner[1,*]

1. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK
2. Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade NOVA de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
3. Medical Research Council Mitochondrial Biology Unit, University of Cambridge, The Keith Peters Building, Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0XY, UK

Supporting Information Placeholder

ABSTRACT: The biological formate hydrogenlyase (FHL) complex links a formate dehydrogenase (FDH) to a hydrogenase (H_2ase) and produces H_2 and CO_2 from formate via mixed-acid fermentation in *Escherichia coli*. Here, we describe an electrochemical and a colloidal semi-artificial FHL system that consists of an FDH and a H_2ase immobilized on conductive indium tin oxide (ITO) as an electron relay. These *in vitro* systems benefit from the efficient wiring of a highly active enzyme pair and allow for the reversible conversion of formate to H_2 and CO_2 under ambient temperature and pressure. The hybrid systems provide a template for the design of synthetic catalysts and surpass the FHL complex *in vivo* by storing and releasing H_2 on demand by interconverting CO_2/H_2 and formate with minimal bias in either direction.

Semi-artificial catalytic systems combine synthetic and biological units to drive challenging reactions and provide new concepts for catalyst design.[1] Such solar-driven systems have already demonstrated coupling of water oxidation to the production of fuels (reduction of protons and CO_2).[2–5] However, storage and transport of energy vectors are also important components in energy production-utilization cycles and their development will benefit from more advanced concepts and model systems.

H_2 is a promising fuel and its storage in formate allows for easier storage and transport; H_2 and formate are therefore an attractive energy vector pair. Furthermore, H_2 gas cleanly separates from dissolved formate, and their interconversion comes at little thermodynamic cost (Eq. 1-3).[6,7] However, achieving kinetic efficiency in HCO_2^-/H_2 interconversion remains a synthetic challenge. Artificial systems commonly compete between decomposition of formic acid to CO and H_2O (dehydration), and CO_2 and H_2 (dehydrogenation), and rely on precious metals, high temperature/pressure, organic solvents and light.[8–10]

$$2\ CO_2 + 2\ H^+ \rightleftharpoons 2\ HCO_2^-\ (E^{0'} = -0.366\ \text{V vs. SHE, pH 6.5}) \quad \text{Eq. 1}$$

$$2\ H^+ \rightleftharpoons H_2\ (E^{0'} = -0.382\ \text{V vs. SHE, pH 6.5}) \quad \text{Eq. 2}$$

$$2\ HCO_2^- \rightleftharpoons 2\ CO_2 + H_2\ (E^{0'}_{rxn} = U^{0'} = -0.016\ \text{V}) \quad \text{Eq. 3}$$

FHL complexes are biological machines for HCO_2^-/H_2 interconversion[11] that are either membrane-associated complexes composed of a multisubunit [NiFe]-H_2ase coupled to a FDH,[11–13] or smaller soluble complexes of an [FeFe]-H_2ase and an FDH.[14,15] The *Escherichia coli* FHL-1 complex, composed of the membrane-bound [NiFe]-H_2ase 3 (HYD-3/HycE) and FDH-H (FdhF; Figure 1a) represents a well-studied FHL, evolving H_2 under fermentative conditions.[11,12] The constituent enzymatic units of FHL-1 have been demonstrated to be reversible electrocatalysts,[16–20] but the complex is catalytically biased toward H_2 production from formate.[14,15,19] Interconversion of HCO_2^-/H_2 has also been reported in whole-cell studies,[14,20] notably in sulfate-reducing bacteria in the absence of sulfate.[21,22] *Desulfovibrio vulgaris* Hildenborough can grow by converting formate to H_2,[23] with formate oxidation catalyzed by a periplasmic FDH, and H_2 produced either via a direct pathway (periplasmic H_2ase) or via transmembrane electron transfer (cytoplasmic H_2ase).[24]

Redox biocatalysts, including H_2ases and FDHs, have been coupled to other enzymatic processes via electron relays. H_2ases have been connected to nitrate and fumarate reductases,[25] diaphorase modules,[26] nicotinamide reductase and alcohol dehydrogenase[27] via graphitic particles. Coupling a H_2ase to carbon monoxide dehydrogenase efficiently catalyzed the water-gas shift reaction.[28] Enzymatic cascades have linked FDH with formaldehyde and alcohol dehydrogenases for methanol production.[29,30] However, the reversible interconversion of substrate and product has not been previously accomplished with such coupled enzymes *in vitro*.

Here, a semi-artificial FHL complex mimic is presented by rewiring FDH[31,32] and H_2ase[33] from *D. vulgaris* Hildenborough into electrochemical and colloidal systems (Figure 1b,c). These systems rely on efficient electrical contact of the [W/Se]-FDH active-site via four [Fe_4S_4] clusters and the [NiFeSe]-H_2ase active-site via three [Fe_4S_4] clusters with nanostructured ITO.

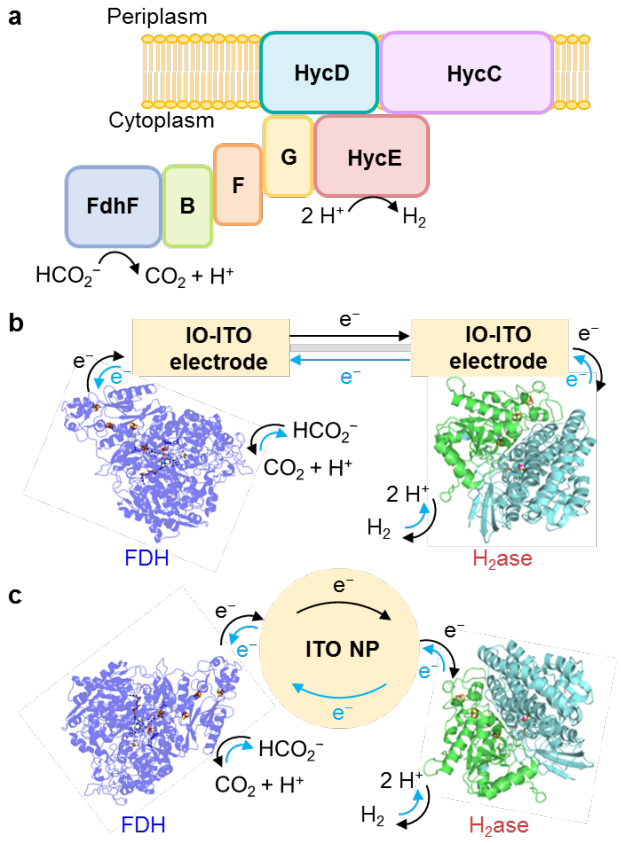

Figure 1. (a) Biological *E. coli* FHL-1 complex. FdhF, [Mo]-FDH; B/F/G, Fe-S cluster-containing proteins; HycE, [NiFe]-H_2ase; HycD/C, membrane proteins.[17] (b) IO-ITO|FDH||IO-ITO|H_2ase cell: IO-ITO|FDH wired to IO-ITO|H_2ase electrode. (c) FDH–ITO–H_2ase nanoparticle (NP) system with enzymes immobilized onto ITO NP in solution. Species size not drawn to scale.

Macro-mesoporous inverse opal (IO) ITO electrodes (20 µm film thickness; 0.25 cm^2 geometrical surface area) were assembled as previously reported.[34] IO-ITO|FDH and IO-ITO|H_2ase electrodes were prepared by drop-casting an FDH solution (2 µL, 19 µM with 50 mM DL-dithiothreitol, incubated for 15 min) and a H_2ase solution (2 µL, 5 µM), onto IO-ITO.[31,34] Protein film voltammetry (PFV) was recorded using a three-electrode configuration (Figure 2a and S1) in CO_2/$NaHCO_3$ solution. Current densities (J) of −185 µA cm^{-2} (CO_2 reduction to formate by FDH) and −450 µA cm^{-2} (H^+ reduction to H_2 by H_2ase) were observed at an applied potential (E_{app}) of −0.6 V vs. standard hydrogen electrode (SHE). Addition of sodium formate (20 mM) to the IO-ITO|FDH system resulted in formate oxidation to CO_2 and 300 µA cm^{-2} was reached at −0.2 V vs. SHE. After purging the IO-ITO|H_2ase system with H_2 (0.4 bar), H_2 oxidation to H^+ was observed and 440 µA cm^{-2} was reached at −0.2 V vs. SHE. The voltammograms cut through zero current around the formal redox potentials (Eq. 1,2), demonstrating reversible electrocatalysis for both enzymes.[6,35]

Multiple PFV scans of IO-ITO|FDH and IO-ITO|H_2ase (Figure S2) showed minimal desorption/activity losses. Controlled-potential electrolysis (CPE) of IO-ITO|FDH and IO-ITO|H_2ase was performed to measure H^+/CO_2 reduction (E_{app} = −0.6 V) as well as H_2/formate oxidation (E_{app} = −0.2 V) (Figure S3). Both electrodes retaining >90% of the initial current after 24 h in both directions. Faradaic efficiencies (η_F) for formate and H_2 production were determined to be 76 and 77%, respectively. Efficiency losses may be attributed to capacitive background current of porous IO-ITO,[34] undetected trapped product and a contribution from ITO/FTO degradation.[36,37]

The comparable formal redox potentials of H^+/H_2 and CO_2/HCO_2^- conversion (Eq. 1-3), reversible catalysis of the individual enzymes, high and matching current densities, and good stability make this enzyme pair a promising candidate for assembling a reversible HCO_2^-/H_2 interconversion system.[6] Thus, the IO-ITO|FDH (working electrode) was wired to the IO-ITO|H_2ase (counter electrode) in a two-electrode configuration (Figure 2b). When no additional substrate was present (only buffering CO_2 and H^+), only a non-catalytic (capacitive) current was observed. Upon addition of formate, an oxidative current was observed (formate oxidation to CO_2 and H^+ reduction to H_2) at a positive applied voltage (U > 0 V); 250 µA cm^{-2} was reached at U = 0.2 V. Addition of H_2 resulted in a reductive current (H_2 oxidation to H^+ and CO_2 reduction to formate) with −250 µA cm^{-2} obtained at U = −0.2 V.

To achieve reversible formate/H_2 interconversion (Eq. 3) both formate and H_2 were added in addition to CO_2 and H^+. A reversible voltammogram was observed, with zero current at approximately $U^{0'}$ at 0.02 V. A marginally more positive or negative voltage drives the reaction in either direction, demonstrating reversible unbiased electrocatalysis, as opposed to that demonstrated for *E. coli* FHL-1.[19] 200 µA cm^{-2} and −200 µA cm^{-2} were reached at U = 0.2 V and −0.2 V, respectively. Multiple PFV scans of the IO-ITO|FDH||IO-ITO|H_2ase cell (Figure S4) showed stability of the system with marginal losses. Control experiments with IO-ITO|FDH (or ITO|H_2ase) wired to IO-ITO (Figure S5) gave only a small capacitive current in the presence and absence of substrates.

CPE during 2 h at U_{app} = 0.2 V with the IO-ITO|FDH||IO-ITO|H_2ase cell with formate present (Figure 2c) produced H_2 (5.84 ± 0.88 µmol cm^{-2}) with η_F of (79 ± 11)%. Similarly, CPE at U_{app} = –0.2 V for 2 h with H_2 present generated formate (5.00 ± 0.80 µmol cm^{-2}) with η_F of (81 ± 15)%. This semi-artificial system exhibited good stability, retaining >95% of its initial activity after 2 h in both directions. After equilibration, the cell exhibited high bidirectional stability for >1 day (Figure S6). For formate oxidation (U_{app} = 0.2 V), H_2 (36.28 µmol cm^{-2}) was detected with η_F = 72%. For H_2 oxidation (U_{app} = –0.2 V), formate (42.80 µmol cm^{-2}) was detected with η_F = 77%. Similarly to the three-electrode systems, capacitive currents and FTO/ITO dissolution[36,37] might have decreased the product yield.

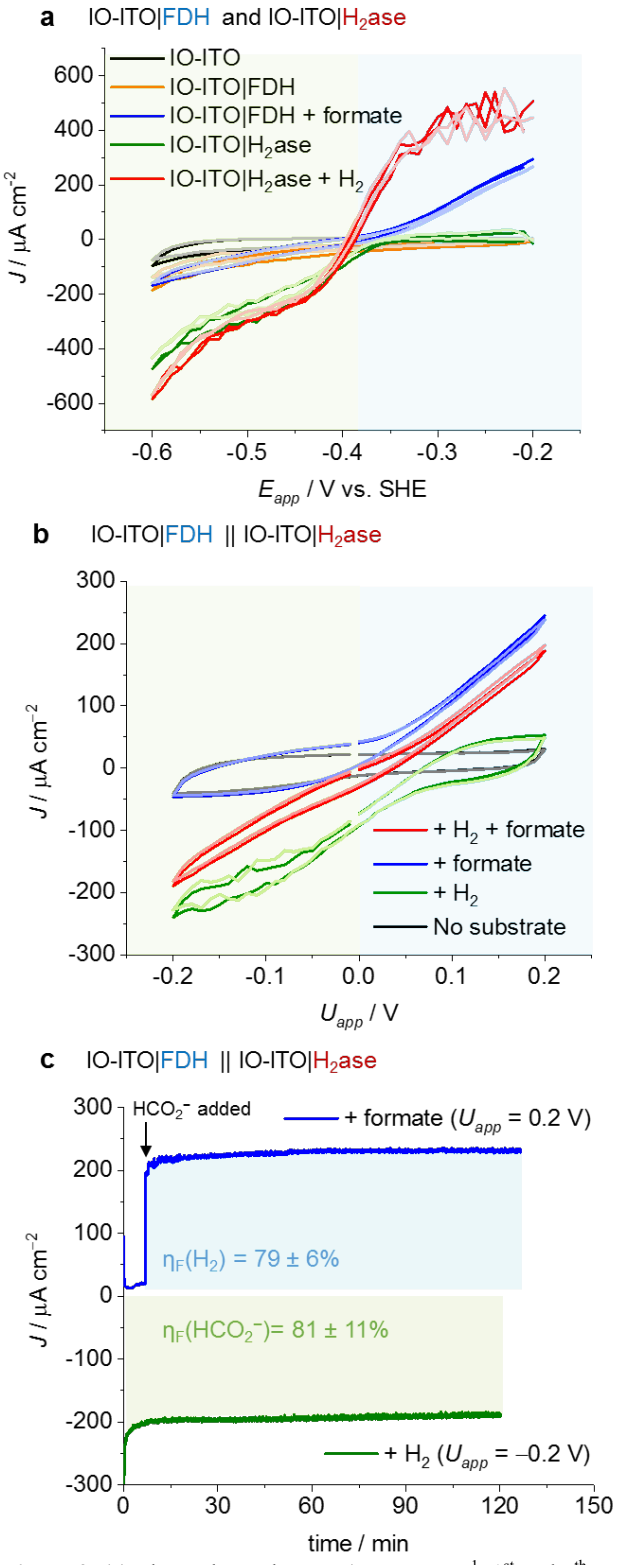

Figure 2. (a) Three-electrode PFV (ν = 5 mV s^{-1}, 1st and 5th scan, increasing transparency) using IO-ITO|FDH or IO-ITO|H_2ase working, Ag/AgCl (KCl_{sat}) reference, Pt counter electrode. (b) Two-electrode PFV (ν = 5 mV s^{-1}, 1st and 5th scan) of IO-ITO|FDH wired to IO-ITO|H_2ase. (c) Two-electrode CPE of IO-ITO|FDH wired to IO-ITO|H_2ase. Conditions: CO_2/$NaHCO_3$ (100 mM), KCl (50 mM), 1 bar CO_2 or 0.4/0.6 bar H_2/CO_2, $pH_{initial}$ = 6.5-6.7, T = 25 °C, stirring. Substrates: formate (20 mM) and/or 0.4/0.6 bar H_2/CO_2.

To further investigate the system's reversibility without electrochemical wiring, FDH and H_2ase were co-assembled on ITO NPs (0.3 mg mL^{-1}) (Figure 3 and S7) dispersed in solution (see Supporting Information). Solutions of FDH (19 nM, incubated as above) and H_2ase (3.4 nM) were added to the vessel, which was sealed and purged with CO_2. Either formate or H_2 was introduced to the vessel. FDH:H_2ase molar ratios (Figure S8) and total concentrations (Figure S9a,b) were screened for optimum H_2 evolution rate. The optimal system contained an enzyme loading of approximately 40 FDH and 7 H_2ase particles per ITO NP, based on the adsorption surface area of 27 m^2 g^{-1}, ~31,400 nm^2 per NP (assuming a 50 nm diameter sphere) and an enzyme footprint of ~100 nm^2.

Upon formate addition to the FDH–ITO–H_2ase system (Figure 3a), H_2 was produced with a rate (Figure S9c) of 0.24 ± 0.01 µmol H_2 h^{-1} during the first 8 h [turnover number, TON = (23.0 ± 1.5)×10^3 and turnover frequency, TOF = 6.4 ± 0.4 s^{-1} for the H_2ase], after which the rate started to decrease (Table S1). Equilibrium was reached after ~72 h (5.82 ± 0.24 µmol H_2, pH 6.88, T = 23 °C), in agreement with calculations (5.95 µmol, 2.97 mM of H_2, see Supporting Information).[7]

In the presence of H_2, the FDH–ITO–H_2ase system (Figure 3b), produced formate with an initial reaction rate of 1.33 ± 0.01 µmol formate h^{-1} [TON = (15.8 ± 5.4)×10^3 and TOF = 4.4 ± 1.5 s^{-1} for the FDH] for the first 8 h (Figure S9d). Equilibrium was reached after ~96 h (36.16 ± 1.47 µmol formate, pH 6.99, T = 23 °C), consistent with calculations (37.11 µmol, 18.56 mM of formate).[7] Control experiments with no ITO NPs, omitting an enzyme or with denatured enzymes (Figure S10) showed only negligible H_2 and formate production (<0.2 µmol) (Table S2 and S3). Therefore, the ITO NPs act as a semi-heterogeneous electron relay facilitating electron transfer between electroactive FDH and H_2ase.

In *D. vulgaris* cells, the two periplasmic enzymes exchange electrons through the type-I cytochrome c_3 (TpIc_3) electron acceptor.[24] We therefore studied the activity of these enzymes in solution with TpIc_3. A high concentration of the cytochrome (1.9 µM, 100-fold excess vs FDH) was required to achieve comparable kinetics of H_2 and formate production (Fig. S11a,b), revealing the superiority of co-immobilizing the two enzymes on synthetic ITO to achieve efficient electron transfer.

In summary, we have presented how semi-artificial systems consisting of FDH and H_2ase from *D. vulgaris* wired to ITO can mimic the biological FHL complex. The semi-artificial FHL systems are based on a bottom-up design that employs a pair of reversible redox enzymes immobilized on conductive scaffolds to enable an overall catalytic reaction to proceed to thermodynamic equilibrium. The semi-artificial FHL concept can be deployed in either an electrochemical cell or a self-assembled colloidal suspension, providing versatility for applications in different contexts. The design concept of linking two half-reactions via a conductive scaffold also provides a blueprint to develop improved synthetic H_2/formate cycling catalysts in future development.

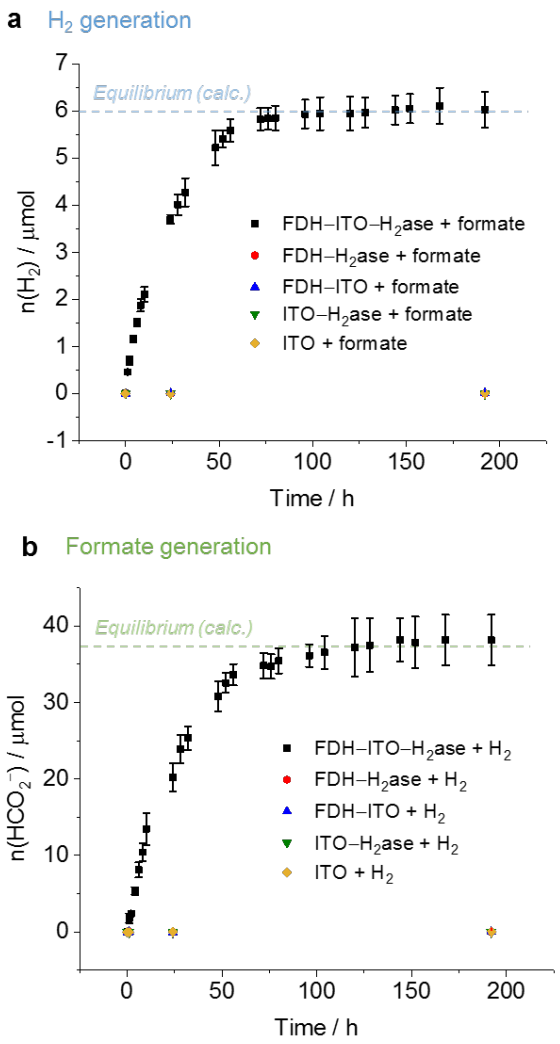

Figure 3. Colloidal FDH–ITO–H_2ase NP system using ITO NPs (0.3 mg mL^{-1}), FDH (19.0 nM) and H_2ase (3.4 nM). (a) H_2 production in the presence of 10 mM formate and 1 bar CO_2. $V_{headspace}$ = 1.72 mL. (b) Formate production in the presence of 0.4/0.6 bar H_2/CO_2. $V_{solution}$ = 2 mL. Conditions: $CO_2/NaHCO_3$ (100 mM), KCl (50 mM), 1 bar CO_2 or 0.4/0.6 bar H_2/CO_2, $pH_{initial}$ = 6.5-6.7, T = 23 °C, stirring.

ASSOCIATED CONTENT

Supporting Information

Materials, experimental methods, Figures and Tables. This material is available free of charge via the ACS Publications website at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

reisner@ch.cam.ac.uk

Author Contributions

‡These authors contributed equally.

Notes

The authors declare no competing interests.

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Table of Contents artwork