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Electroenzymatic C-C bond formation from CO_2

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Supporting Information Placeholder

ABSTRACT: Over the last decade, there has been significant research in electrochemical reduction of CO_2, but it has been difficult to develop catalysts capable of C-C bond formation. Here, we report bioelectrocatalysis of vanadium nitrogenase from *Azotobacter vinelandii*, where cobaltocenium derivatives transfer electrons to the catalytic VFe protein, independent of ATP-hydrolysis. In this bioelectrochemical system, CO_2 is reduced to ethylene (C_2H_4) and propene (C_3H_6), by a single metalloenzyme.

Due to ever-increasing carbon dioxide (CO_2) emissions and environmental concerns, electrochemical CO_2 reduction to commodity chemicals, or electrofuels, such as formate ($HCOO^-$) and methane (CH_4) is a prominent theme among electrochemical researchers in attempts to close the anthropogenic carbon cycle. The conversion of CO_2 to hydrocarbons has been of interest for many decades;[1] however, the formation of hydrocarbons from CO_2 involving multiple hydrogenations, electron transfers and C-C coupling remains challenging.[2] Chemical processes are used predominantly in industry for the hydrogenation of CO_2 and carbon monoxide (CO), such as the Sabatier or Fischer-Tropsch reactions, although many require high temperatures (453~673K) and high pressures (≥ 7 bar).[3]

While many promising electrocatalysts are currently being explored for such reactions, the majority of aqueous systems are plagued by poor selectivity over the hydrogen evolution reaction (HER), with molecular hydrogen (H_2) often being the major product. [4]Perhaps the most promising CO_2 reduction reaction (CO_2RR) catalysts are derived from Cu.[2, 5] In contrast to metallic and molecular catalysts, multiple enzymatic systems have been employed for bioelectrocatalytic CO_2RR.[4b] Carbon monoxide dehydrogenase (CODH) is a metalloenzyme that interconverts CO and CO_2, where direct bioelectrochemistry has been reported.[6] Nicotinamide adenine dinucleotide-dependent (NAD) enzymes have also been utilized for CO_2, where an enzymatic cascade was able to reduce CO_2 to methanol (CH_3OH).[7] Mo- and W-dependent formate dehydrogenases do not employ diffusive NAD cofactors (thereby negating the need to separate/refresh the cofactor from the substrate/products), and as such, their bioelectrochemical properties have also been exploited for the reduction of CO_2 to formate ($HCOO^-$).[8]

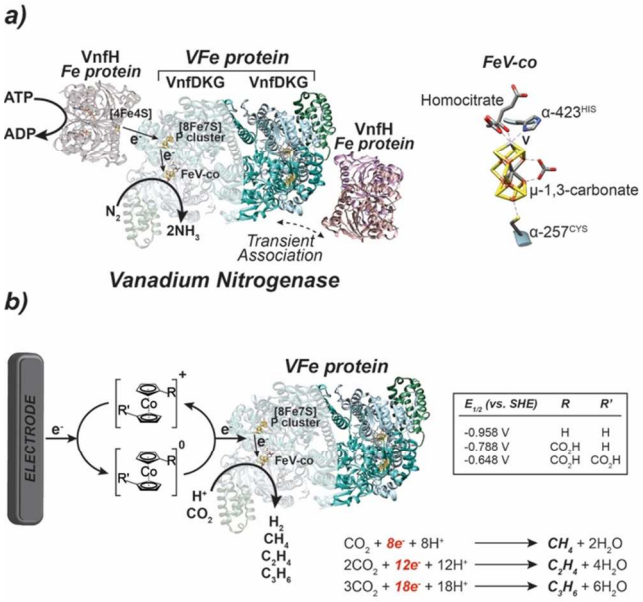

Figure 1. a) Crystal structure of vanadium nitrogenase and FeV-co from *Azotobacter vinelandii*, depicting the transient association of the VnfH Fe protein with the catalytic VnfDKG VFe protein. The crystal structure of the VFe protein was adapted from PDB file 5N6Y and the Fe protein for molybdenum nitrogenase (NifH) was used to depicted the VnfH Fe protein, whose structure has not yet been determined (PDB file 4WZA). **b)** Bioelectrocatalytic turnover of VFe nitrogenase mediated by derivatives of cobaltocene/cobaltocenium.

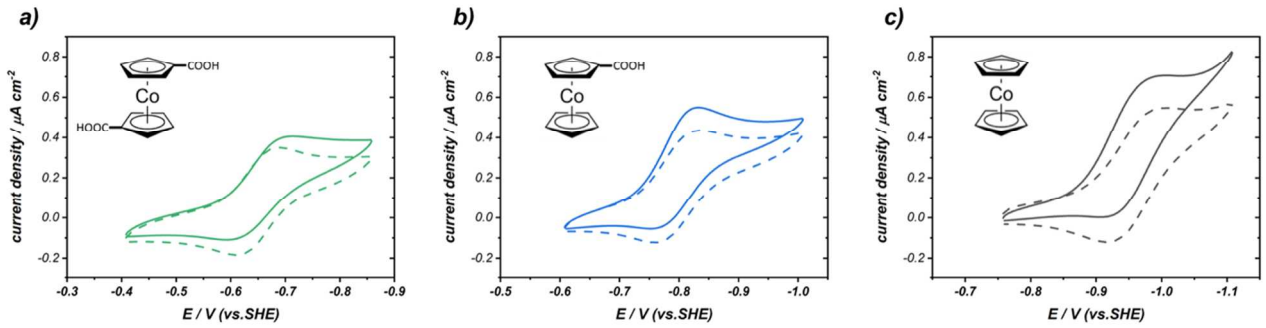

Figure 2. Representative cyclic voltammograms of VFe bioelectrocatalysis of H^+ reduction using **(a)** $Cc(CO_2H)_2$, **(b)** $Cc(CO_2H)$ or **(c)** Cc to mediate electrons between the electrode and the VFe protein. In all cases, the dashed lines were recorded in the absence of the VFe protein and the solid lines represent the bioelectrocatalytic response obtained following the addition of VFe protein to the electrolyte. Measurements were performed at 2 mV/s in a stationary solution of 100 mM MOPS buffer (pH 7.0), under anoxic conditions (O_2 < 1 ppm). The working electrode was a glassy carbon electrode with a geometric surface area of 0.071 cm^2.

In contrast to the CO_2RR, the nitrogen reduction reaction (NRR) of molecular nitrogen (N_2) to ammonia (NH_3) has gained attention in the realm of bioelectrocatalysts, where the only enzyme known to reduce molecular nitrogen (N_2) to NH_3 has recently been interfaced with electrodes for applied and mechanistic evaluation.[9] Nitrogenases are essential enzymes whose primary physiological function is to catalyze the reduction of N_2 to NH_3 at the expense of ATP-hydrolysis, allowing some microbes to grow diazotrophically.[10] All nitrogenases are a bi-enzyme cascade that consist of a reducing component protein (Fe protein) and one of three catalytic proteins, which are characterized by their molybdenum-iron (MoFe)-, vanadium-iron (VFe)- or iron-only (FeFe)-dependent catalytic cofactors.[11] MoFe nitrogenase is commonly recognized as the "conventional" nitrogenase, while VFe nitrogenase (VFe) and Fe nitrogenase (FeFe) are considered as alternative nitrogenases that are expressed under Mo starvation.[12]

VFe nitrogenase was first isolated in 1986 by Hales et al.[13] VFe nitrogenase is encoded by the *vnf* operon, which contains genes for its own Fe protein (VnfH) alongside the catalytic VFe protein. In contrast to the MoFe nitrogenase heterotetramer (a dimer of dimers), VFe nitrogenase is a heterohexamer (a dimer of trimers) containing an additional subunit encoded by the *vnfG* gene ($VnfD_2K_2G_2$, Figure 1a).[12] It is assumed that VFe nitrogenase follows a similar catalytic mechanism to that of MoFe nitrogenase, whereby the reduced Fe protein binds to the catalytic VFe protein and a single-electron transfer (ET) from the Fe protein is coupled to the hydrolysis of ATP, before dissociation of the transient VFe-Fe protein complex.[14] Despite these assumed similarities, the recently-determined X-ray crystal structure of VFe nitrogenase also revealed the presence of an unusual bridging ligand at the FeV-cofactor (currently thought to be a μ-1,3-carbonate, Figure 1a).[15] Excitingly, the wild-type VFe nitrogenase has also been demonstrated to be able to undergo Fischer-Tropsch chemistry at ambient pressure and temperature, producing up to C_3 products from CO *in vivo* (in *Azotobacter vinelandii*) and *in vitro*.[14, 16]

Here, we show that a mediated electrochemical approach can be used to interface VFe nitrogenase with an electrode, whereby bioelectrocatalytic turnover can be realized in the absence of the VnfH Fe protein and ATP-hydrolysis. Initially, we report the use of 2 additional cobaltocene electron mediators to the previously employed unsubstituted cobaltocene used for MoFe nitrogenase bioelectrocatalysis. Finally, we demonstrate that this bioelectrocatalytic VFe system can reduce CO_2 to ethylene (C_2H_4) and propene (C_3H_6), bypassing the requirement of CO as the substrate and forming C-C bonds. Importantly, significant quantities of $C_{>1}$ products (C-C bond formation) have not been observed for wild-type MoFe and FeFe nitrogenases.[9e] We highly anticipate that this bioelectrocatalytic approach to contacting VFe nitrogenase will be essential in revealing further mechanistic details that may lead to improve biological and molecular electrocatalysts for CO_2RRs and C-C bond formation.

We previously reported that the cobaltocene/cobaltocenium (Cc^+ PF_6^-) redox couple ($E_{1/2}$ = -0.958 vs. SHE) was able to support mediated electron transfer (MET) to MoFe nitrogenase in the absence of the Fe protein/ATP-hydrolysis, where the bioelectroreduction of $2H^+$ to H_2, NO_2^- to NH_3 and N_3^- to NH_3 was observed. We evaluated whether Cc could act as an electron mediator to VFe nitrogenase in addition to 2 other Cc derivatives, 1,1'-dicarboxy-cobaltocenium ($Cc(CO_2H)_2$), 1-carboxy-cobaltocenium ($Cc(CO_2H)$) (Figure 1b). The cobaltocene derivatives have more-positive reduction potentials than the original cobaltocene mediator. We hypothesized that these cobaltocene derivatives could still mediate electron transfer between the VFe protein and an electrode for carbon dioxide reduction products (formal potentials for CO_2/CH_4, $2CO_2/C_2H_4$, and $3CO_2/C_3H_6$ are calculated as -0.24, -0.34, and -0.31 V vs. SHE at pH = 7), while minimizing the potential difference (overpotential) required for bioelectrocatalysis. Additionally, the P cluster is estimated to be located ~12 Å within the VFe protein (from the surface), whereas the FeV-co is estimated to be located at ~15 Å. This makes the possibility of contacting VFe by direct electron transfer unlikely where physiologically-relevant rates of biological electron transfer commonly occur across distances of less than 14 Å.[17]

Initially, we evaluated the possibility of mediating electron transfer between an electrode and the VFe protein by these Cc derivatives under anaerobic, stationary conditions (Figure 2). Since wild-type nitrogenases revert to 100 % H^+ reduction in the absence of other substrates, proton reduction was initially explored and the absence of VFe served as the control experiments. In the case of all three Cc derivatives, an apparent reductive catalytic response was observed following the addition of VFe (52 μg) to the electrolyte. Interestingly, these initial results indicated that the apparent onset potential for

bioelectrocatalysis can be shifted more positively by ~200 mV when $Cc(CO_2H)_2$ is used in the place of the Cc electron mediator first explored with the MoFe protein, with a reductive catalytic current appearing at an approximate onset of -0.65 V vs. SHE. Offering a promising onset potential of bioelectrocatalysis, the $Cc(CO_2H)_2$ derivative was selected for subsequent analysis.

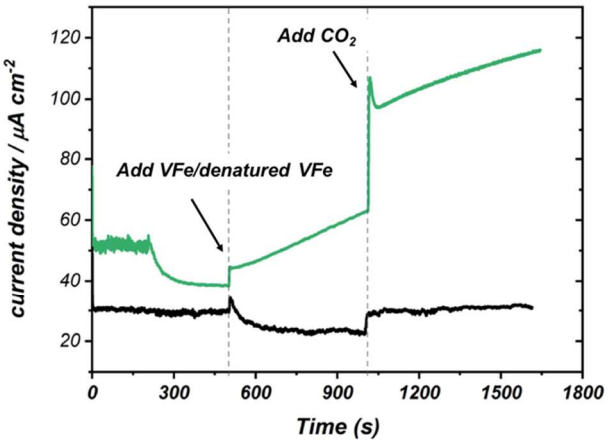

Figure 3. Amperometric i-t trace for $Cc(CO_2H)_2$-mediated bioelectrocatalytic reduction of CO_2 by VFe nitrogenase. The experiments were performed in a stirred 2 mL solution of 100 μM $Cc(CO_2H)_2$ in MOPS buffer (pH 7.0, 100 mM) at an applied potential of -0.86 V vs. SHE, using a custom-made H-cell with a headspace of 7 mL. Initially (green trace), denatured VFe protein (1.3 mg) was added at 200 s, followed by active VFe protein (1.3 mg) at 500 s. Finally, 1 mL of CO_2 was added at 1000 s. The experiment (black trace) was performed with only denatured VFe protein (1.3 mg) added at 500 s followed by 1 mL CO_2 at 1000 s.

Additional control experiments were performed by the sequential addition of an equivalent amount of catalytically-inert bovine serum albumin (BSA) in 2 mM sodium dithionite (to account for the buffer and reducing agent in which VFe was purified) or denatured VFe (where VFe was exposed to air for 30 min) before adding active VFe (Figure S1). Interestingly, increasingly reductive currents were observed following the sequential additions of BSA (in 2 mM DT) and denatured VFe, although the reductive response increased further following the addition of active VFe. As a catalytically-inert protein, BSA does not contribute towards any reductive current. Our original bioelectrochemical study of MoFe nitrogenase highlighted that an apo-MoFe protein variant (deficient in FeMo-co and containing only the P cluster) yielded a diminished reductive current implying that the P cluster has minimal bioelectrocatalytic activity, thus, we hypothesize that the reductive current observed in the denatured VFe control is due to persistent bioelectrocatalytic activity of the P cluster for H^+ reduction (Figure S2).[9a] Further, premature FeMo-co assembly proteins are reported to be able to undergo the acetylene reduction reaction (which is synonymous to the NRR and is used to prove nitrogenase activity) and the observed electrochemical activity may be due to partially-disassembled P clusters or FeV-co.[18]

To further validate the bioelectrocatalytic activity of VFe nitrogenase, steady-state amperometric analyses were conducted with $Cc(CO_2H)_2$ at an applied potential of -0.86 V *vs.* SHE (Figure 3). In addition to the expected reduction of H^+ to H_2, CO_2 was included as a substrate to evaluate the product distribution of VFe nitrogenase in a bioelectrochemical setting. Directly after the addition of VFe, a significant increase in reductive current was observed, reflecting the bioelectrocatalytic reduction of H^+ in the absence of CO_2. Importantly, no reductive signal was observed following the addition of denatured VFe under these experimental conditions. A further

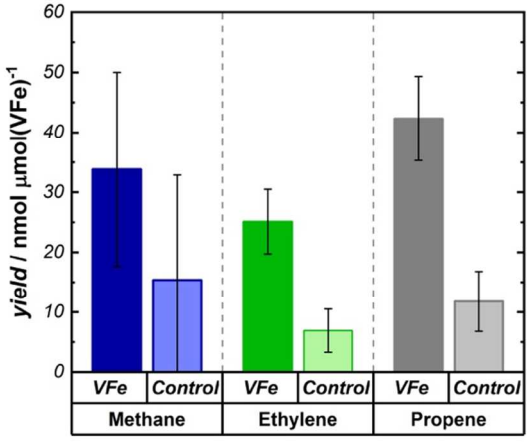

Figure 4. Product distribution of the bioelectrocatalytic CO_2RR by VFe nitrogenase, demonstrating the production of CH_4 (blue), C_2H_4(green) and C_3H_6 (grey). Control experiments were performed with VFe that had been denatured by exposure to air for 30 minutes. Error bars represent one standard deviation (n = 3).

increase (~150 %) in the reductive signal was observed following the addition of 1 mL CO_2 in the presence of the native VFe, indicating the bioelectrocatalytic reduction of CO_2. Once again, no bioelectrocatalytic signal was observed when denatured VFe was added in the place of VFe.

Finally, bulk bioelectrosynthesis and determination of the product distribution of the CO_2RR was performed to confirm the bioelectrocatalytic activity of VFe, by gas chromatography (Figure 4). Bulk bioelectrosynthesis experiments were carried out in a custom-made sealed H-cell. Control experiments were performed with denatured VFe and blank experiments were performed with active VFe protein in the absence of CO_2. The products were detected and quantified after the passage of 4 C of charge (ca. 20 h) at -0.86 V (*vs.* SHE). Triplicate experiments revealed the production of 25 ± 5 nmol C_2H_4 and 42 ± 7 nmol C_3H_6 per μmol VFe (corrected to blank experiments and statically significant at the 95% confidence level), demonstrating that the bioelectrocatalytic reduction of CO_2 by the VFe nitrogenase protein results in the reductive formation of C-C bonds, presenting an alternative approach to the electrosynthesis of $C_{\geq 2}$ products. Interestingly, no carbon monoxide (CO) was detected in this system, which may indicate that the initial reduction of CO_2 may be rate-limiting where $C_{\geq 2}$ hydrocarbon formation from CO_2 is not favorable and presumably takes place *via* the CO produced from CO_2 reduction.[19]

In conclusion, we report that the VFe protein of vanadium nitrogenase can be electronically contacted by the use of Cc derivatives as electron mediators, where VFe nitrogenase electrochemistry has not yet been reported. While the production of $C_{\geq 2}$ hydrocarbons by VFe nitrogenase has been demonstrated, we report that an electrochemical approach can be used to regenerate reducing equivalents in the place of the

natural VFe partner, VnfH (Fe protein), presenting a novel bioelectrochemical approach for the formation of C-C bonds. We anticipate that this bioelectrochemical approach will be utilized to reveal further mechanistic details surrounding the formation of $C_{\geq 2}$ hydrocarbons from CO_2 by VFe, including the determination of the full substrate/product distribution for VFe bioelectrocatalysis.

ASSOCIATED CONTENT

Supporting Information

Materials, *A. vinelandii* growth, protein purification, activity assays, electrochemical experimentation, gas chromatography experimental, control experiments.The Supporting Information is available free of charge on the ACS Publications website.

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Notes

There are no conflicts to declare. Electrochemical experiments were performed against a saturated calomel electrode (SCE) and reported against the standard hydrogen electrode (SHE), corrected by the following relationship: $E_{(SCE)} = E_{(SHE)} - 0.242$ V.[20]

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