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Communication—CO_2 Reduction to Formate: An Electro-Enzymatic Approach Using a Formate Dehydrogenase from *Rhodobacter capsulatus*

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CO_2 utilization for producing value-added chemicals has recently emerged as a strategy to mitigate atmospheric CO_2 levels. Given that (i) certain formate dehydrogenases are capable of interconverting CO_2 and formate, and (ii) formate is versatile in various industries, we, herein, aimed to demonstrate FDH-driven formate production from CO_2. Because of its O_2 stability, we selected FDH from *Rhodobacter capsulatus* (RcFDH) and then constructed a mediated electro-enzymatic system. The mediated electro-enzymatic kinetic parameters (k_{red} and k_{ox}) were calculated to optimize the reaction conditions favorable for CO_2 reduction. Finally, a RcFDH-driven electro-enzymatic system successfully produced 6 mM of formate in 5 hours.
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The combustion of fossil fuels has accelerated the accumulation of atmospheric CO_2 and causes climate change and global warming. Thus, recent studies have focused on diminishing atmospheric CO_2 levels via the capture, storage, and utilization of CO_2.[1] Among the strategies, CO_2 utilization is emerging for producing useful fuels and chemicals from CO_2 as a cheap, abundant, and renewable feedstock.[2] Various fuels and chemicals can be obtained from CO_2 (e.g., CO, methanol, and hydrocarbon), and formate is of primary interest because of its versatility. Formate has been used not only in various industrial applications[3] but also as a key platform chemical for further applications such as a carbon source for formatotrophs[4] and fuel for a formate fuel cell.[5]

Chemical and electrochemical methods have been attempted to convert CO_2 to formate, but practical applications are still limited. CO_2 conversion to formate is usually performed under harsh conditions (i.e., high temperature and pressure),[6] and by-products (e.g., hydrogen, CO, and ethylene) are frequently produced, resulting in low selectivity.[7] Given that a biocatalyst usually functions under moderate conditions (i.e., neutral pH, ambient temperature, and atmospheric pressure) and has higher substrate specificity, the enzymatic CO_2 conversion to formate might be a potential alternative.

Although formate dehydrogenase (FDH, E.C. 1.2.1.2) usually catalyzes formate oxidation to CO_2, certain FDHs are capable of reversibly interconverting CO_2 and formate.[8–10] Hirst et al. reported that the tungsten-containing formate dehydrogenase 1 from *Syntrophobacter fumaroxidans* (SfFDH)[8] and the molybdenum-containing formate dehydrogenase H from *Escherichia coli* (EcFDH)[9] are electroactive and can catalyze CO_2 reduction to formate. However, SfFDH and EcFDH function under strict anaerobic conditions, thereby restrictive applications.[8,9] Thus, we focused on FDH from *Rhodobacter capsulatus* (RcFDH), a kind of molybdenum-containing FDH. Hartmann et al. reported that RcFDH is NAD-linked and catalyzes both CO_2 reduction and formate oxidation under aerobic conditions.[10]

Herein, we aimed to develop a RcFDH-driven formate production system utilizing CO_2 and thus constructed by a mediated electro-enzymatic system (Scheme 1). To optimize a mediated electro-enzymatic system favorable for CO_2 reduction rather than for formate oxidation, we calculated electro-enzymatic kinetic constants (k_{red} and k_{ox}) for various pH and electron mediators based on the limiting current measured by cyclic voltammetry (CV). Furthermore, we successfully achieved measurable formate production from CO_2 under aerobic conditions in our optimized electro-enzymatic system.

Experimental

RcFDH was expressed as previously described,[10] and purified as the following the steps. The cell pellet was suspended in Bugbuster Master Mix (Merck) solution, and then cell debris was removed by centrifugation. Ni-NTA agarose (QIAGEN) was added to the cell lysate, and the mixture was incubated in ice. The mixture was loaded into the column and the resin was washed with washing buffer (50 mM $NaHPO_4$, 300 mM NaCl, 20 mM imidazole, pH 8.0). The bound RcFDH was eluted with elution buffer (50 mM $NaHPO_4$, 300 mM NaCl, 250 mM imidazole, pH 8.0). The elution buffer was replaced with storage buffer (75 mM potassium phosphate, 10 mM KNO_3, pH 7.5) by Amicon Ultra centrifugal filters (50 kDa, Merck). The formate oxidation activity of RcFDH was measured with 2 ml of 100 mM Tris-HCl buffer (pH 9.0) containing 6 mM sodium formate and 2 mM NAD^+ at 25°C. The activity was calculated by the increase in the absorbance of NADH at 340 nm with a UV-spectrophotometer (ε_{NADH}: 6,220 $M^{-1}cm^{-1}$). All CV measurements were carried out at a 10 mV/s scan rate in 10 ml of argon-saturated 100 mM potassium phosphate buffer (KPB) at 30°C, containing 100 mM potassium bicarbonate and potassium formate for bicarbonate reduction and formate oxidation, respectively. 44 μM of alizarin red S (ARS), anthraquinone-2-sulfonic acid (AQ2S), benzyl viologen (BV), and methyl viologen (MV) were tested to determine the most effective electron mediator for RcFDH-driven CO_2 reduction. In the following equations, (i) $k_{cat,\ red}$, $k_{cat,\ ox}$ and $i_{lim,\ red}$, $i_{lim,\ ox}$ are the catalytic constants and the limiting currents for bicarbonate reduction and formate oxidation, respectively, (ii) $K_{M,\ red}$, $K_{M,\ ox}$ and $C_{M,\ red}$, $C_{M,\ red}$ are the Michaelis-Menten constants and concentrations for the reduced and oxidized electron mediators, respectively, (iii) n_M, $n_{HCO_3^-}$, and n_{HCOO^-} are the numbers of electrons in the mediator, HCO_3^-, and $HCOO^-$, respectively, and (iv) F, A, and D_M are the Faraday constant, the electrode surface area, and diffusion constants of the mediator, respectively.[11]

$$k_{red} \equiv \frac{k_{cat,\ red}}{K_{M,\ red}} = \frac{\left(\frac{i_{lim,\ red}}{FAC_{M,\ ox}}\right)^2}{n_{HCO_3^-} D_M C_E}$$

$$k_{ox} \equiv \frac{k_{cat,\ ox}}{K_{M,\ ox}} = \frac{\left(\frac{i_{lim,\ ox}}{FAC_{M,\ red}}\right)^2}{n_{HCOO^-} D_M C_E}$$

The mediated electro-enzymatic CO_2 reduction was performed in an electrochemical reactor, in which the anodic and cathodic compartments were separated by a proton exchange membrane (Nafion115, DuPont). The anodic compartment was equipped with Pt wire and filled with 1 mM H_2SO_4. A glassy carbon plate (2.0 cm × 1.5 cm) and an Ag/AgCl electrode were placed in the cathodic compartment that contained 10 ml of 200 mM KPB, 10 mM KNO_3, 10 mM MV, and

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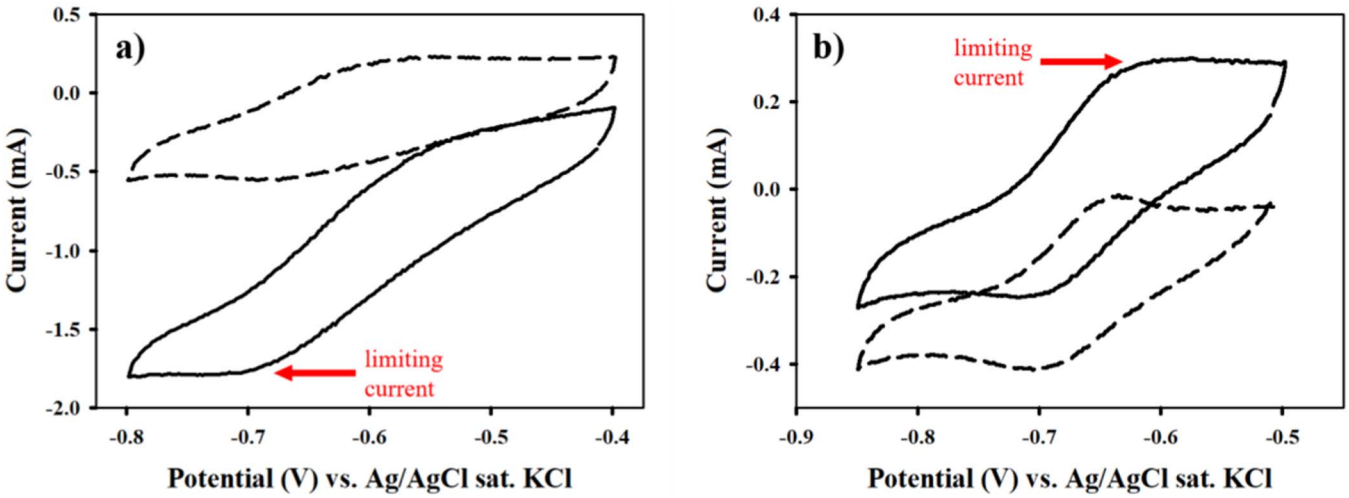


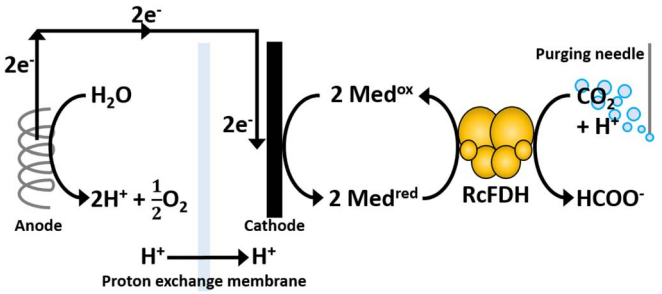
Figure 1. Cyclic voltammograms of 44 μM MV without RcFDH (dashed line), and with RcFDH (solid line) at a glassy carbon electrode in the 100 mM KPB at 30°C (scan rate: 10 mV/s). a) 100 mM potassium bicarbonate reduction at pH 7.0. b) 100 mM potassium formate oxidation at pH 7.0.

65 U of RcFDH. CO_2 gas (99.999%) was purged into the cathodic compartment at a flow rate of 1.0 ml/s. Formate was quantified by a HPLC (Agilent 1200 series) equipped with an Aminex HPX 87-H column (300 × 7.8 mm, Bio-Rad) and a refractive index detector.[2]

Results and Discussion

As previously reported, we successfully obtained a heterologously expressed recombinant RcFDH in *Escherichia coli* and purified it under aerobic condition (Fig. S1). In order to calculate the amount of catalytically active RcFDH, reduction spectra were recorded as shown in Fig. S2, thereby indicating that the purified recombinant RcFDH was fully active. When using sodium formate as the substrate, k_{cat} and K_M of the purified recombinant RcFDH were determined to be 7,440.4 min^{-1} and 237.5 μM, respectively (Fig. S3). Fig. 1 indicates that the reduction and oxidation peak potential of MV were determined at −0.68 V (vs. Ag/AgCl, dashed line in Fig. 1a) and −0.64 V (vs. Ag/AgCl, dashed line in Fig. 1b), respectively. The limiting current plateau of the mediated electro-enzymatic bicarbonate reduction and formate oxidation were observed by adding RcFDH at potentials below of −0.7 V (vs. Ag/AgCl, solid line in Fig. 1a) and above of −0.6 V (vs. Ag/AgCl, solid line in Fig. 1b), respectively. Based on the mediated electro-enzymatic kinetic parameters introduced by Sakai et al.,[11] we calculated the kinetic parameters of the RcFDH-driven CO_2 reduction ($k_{red} \equiv k_{cat,\ red}/K_{M,\ red}$) and the formate oxidation ($k_{ox} \equiv k_{cat,\ ox}/K_{M,\ ox}$).

We tested MV, BV, ARS, and AQ2S to determine the most effective electron mediator for RcFDH-driven CO_2 reduction. Fig. 2a shows that MV was more efficient for CO_2 reduction than other mediators at pH 7.0, and the parameters k_{red} was larger than k_{ox} for MV. Thus, we selected MV as the most suitable electron mediator for the electro-enzymatic CO_2 reduction. Sakai et al. previously reported that a correlation between formal potential of the mediators and log*k* values in the MeFDH-driven electro-enzymatic reaction.[11] In that paper, $\log k_{red}$ was linearly decreased with the formal potential of the



Scheme 1. Schematic illustration of a mediated electro-enzymatic system for RcFDH-driven formate production from CO_2.

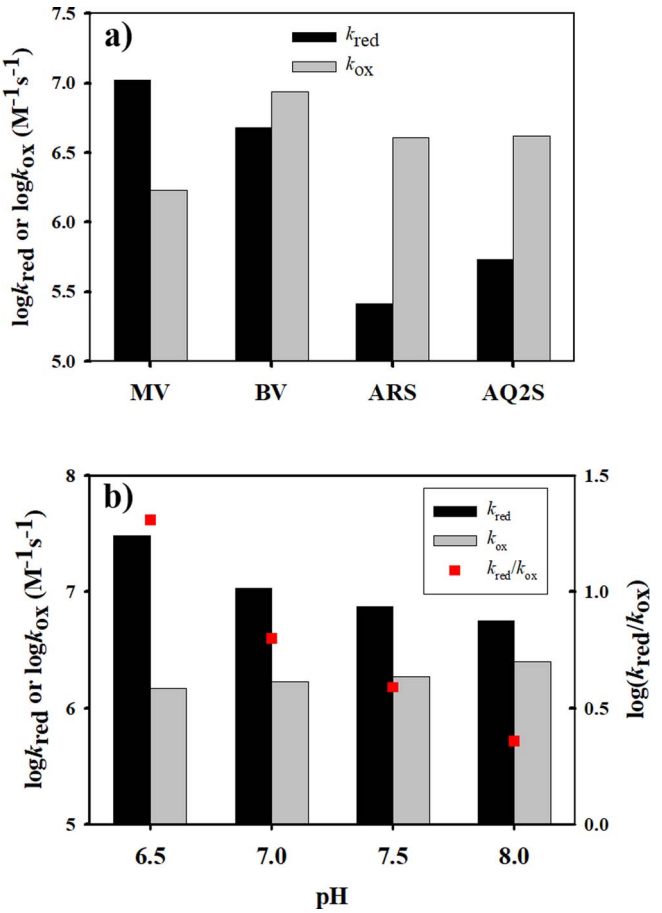


Figure 2. Logarithmic values of kinetic constants between RcFDH and electron mediators. a) Effect of mediators on the kinetic constants; MV, BV, ARS, and AQ2S. b) Effect of pH on the kinetic constants; pH range of 6.5–8.0. k_{red}: kinetic constant of bicarbonate (CO_2) reduction; left *y*-axis (■), k_{ox}: kinetic constants of formate oxidation; left *y*-axis (■), and k_{red}/k_{ox}; right *y*-axis (■).

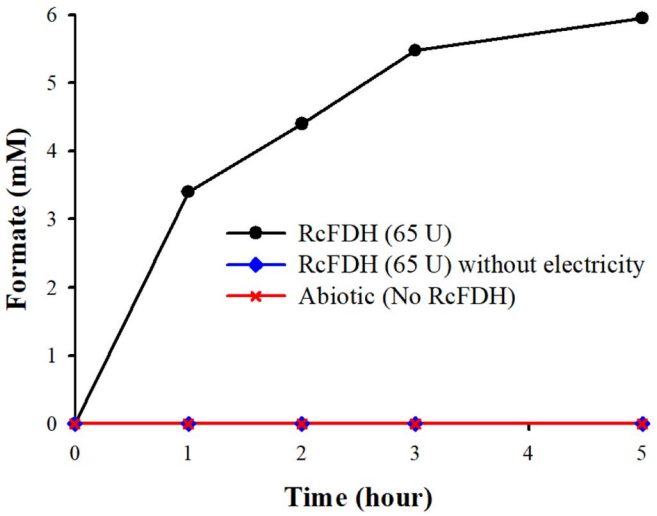


Figure 3. Formate production from CO_2 in the mediated electro-enzymatic system (200 mM KPB containing 10 mM KNO_3, pH 7.0). 10 mM of MV was used as the electron mediator.

mediator, and it was also diminished with the reduction peak potential of the mediator. However, we did not observe a relationship between logk_{red} and the reduction peak potential (the reduction and oxidation peak potentials of each mediator are summarized in Table S1), probably because of structural differences (e.g., Mo-containing for RcFDH and W-containing for MeFDH) that might lead to mediator preference under different circumstances.[12–14]

We investigated the effect of pH on the mediated electro-enzymatic system. The RcFDH-driven CO_2 reduction was more predominant at an acidic pH, whereas formate oxidation was favorable at a basic pH (Fig. 2b). This phenomenon might be due to concentrated protons (H^+), which are inevitably required to convert CO_2 to formate, thereby favoring an acidic pH. On the other hand, formate oxidation to CO_2 releases protons and easily occurs at a basic pH. Accordingly, the ratio of k_{red} to k_{ox} (■ in Fig. 2b) implied that pH 6.5 was the most suitable for CO_2 reduction in our mediated electro-enzymatic system.

Nevertheless, RcFDH had low stability at an acidic pH, especially at pH 6.0, retaining 81% of the initial activity after 7 hours (Fig. S4a) and thus RcFDH-driven CO_2 reduction reaction was performed at pH 7.0. In addition, nitrate enhanced the stability of RcFDH (Fig. S4b), with 10 mM of KNO_3 employed as the stabilizing additive in the mediated electro-enzymatic system.[15]

Finally, RcFDH-driven formate production from CO_2 was performed under optimized conditions with MV as the electron mediator at pH 7.0. When using 65 U of RcFDH, we successfully achieved 6 mM of formate production in 5 hours. The current density decreased from initially 0.65 mA/cm^2 to finally 0.55 mA/cm^2, consistent with the decline of formate production rate in Fig. 3, because probably the activity of RcFDH decreased under electrochemical conditions. In an abiotic system as a negative control, no formate production was observed (× in Fig. 3), even though sufficient CO_2 and reduced MV were provided in the reaction solution. To the best of our knowledge, this is the first report of measurable FDH-driven formate production from CO_2.

Summary

Herein, we constructed a mediated electro-enzymatic system for producing formate from CO_2 as a sustainable feedstock. Based on the electro-enzymatic kinetic constants (k_{red} and k_{ox}), the system was optimized with MV at pH 6.5. Finally, RcFDH-driven CO_2 reduction resulted in 6 mM of formate in 5 hours under aerobic condition. Consequently, the results discussed herein not only might open up the next door to produce the versatile chemical formate from CO_2 as a cheap, abundant, and renewable feedstock but also might contribute to mitigate the atmospheric CO_2 level as a climate change mitigation strategy.

Acknowledgments

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References

1. A. J. Hunt, E. H. K. Sin, R. Marriott, and J. H. Clark, *ChemSusChem*, **3**, 306 (2010).
2. H. Hwang, Y. J. Yeon, S. Lee, H. Choe, M. G. Jang, D. H. Cho, S. Park, and Y. H. Kim, *Bioresour. Technol.*, **185**, 35 (2015).
3. W. Reutemann and H. Kieczka, *Ullmann's Encycl. Ind. Chem*, p. 1, Wiley-VCH Verlag GmbH & Co. KGaA, Germany (2000).
4. O. Yishai, S. N. Lindner, J. Gonzalez de la Cruz, H. Tenenboim, and A. Bar-Even, *Curr. Opin. Chem. Biol.*, **35**, 1 (2016).
5. C. Rice, S. Ha, R. I. Masel, P. Waszczuk, A. Wieckowski, and T. Barnard, *J. Power Sources*, **111**, 83 (2002).
6. A. Azua, S. Sanz, and E. Peris, *Chem.-Eur. J.*, **17**, 3963 (2011).
7. R. Kortlever, K. H. Tan, Y. Kwon, and M. T. M. Koper, *J. Solid State Electrochem.*, **17**, 1843 (2013).
8. T. Reda, C. M. Plugge, N. J. Abram, and J. Hirst, *Proc. Natl. Acad. Sci. U. S. A.*, **105**, 10654 (2008).
9. A. Bassegoda, C. Madden, D. W. Wakerley, E. Reisner, and J. Hirst, *J. Am. Chem. Soc.*, **136**, 15473 (2014).
10. T. Hartmann and S. Leimkühler, *FEBS J.*, **280**, 6083 (2013).
11. K. Sakai, B.-C. Hsieh, A. Maruyama, Y. Kitazumi, O. Shirai, and K. Kano, *Sens. Bio-Sensing Res.*, **5**, 90 (2015).
12. D. R. Jollie and J. D. Lipscomb, *Methods Enzymol.*, **188**, 331 (1990).
13. H. G. Enoch and R. L. Lester, *J. Biol. Chem.*, **250**, 6693 (1975).
14. A. KrÖGer, E. Winkler, A. Innerhofer, H. Hackenberg, and H. SchÄGger, *Eur. J. Biochem.*, **94**, 465 (1979).
15. J. Friedebold and B. Bowien, *J. Bacteriol*, **175**, 4719 (1993).