Interconversion between formate and hydrogen carbonate by tungsten-containing formate dehydrogenase-catalyzed mediated bioelectrocatalysis

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A B S T R A C T

We have focused on the catalytic properties of tungsten-containing formate dehydrogenase (FoDH1) from Methylobacterium extorquens AM1 to construct a bioelectrochemical interconversion system between formate (HCOO−) and hydrogen carbonate (HCO3−). FoDH1 catalyzes both of the HCOO oxidation and the HCO3 reduction with several artificial dyes. The bi-molecular reaction rate constants between FoDH1 and the artificial electron acceptors and NAD+ (as the natural electron acceptor) show the property called a linear free energy relationship (LFER), indicating that FoDH1 would have no specificity to NAD+. Similar LFER is also observed for the catalytic reduction of HCO3−. The reversible reaction between HCOO− and HCO3− through FoDH1 has been realized on cyclic voltammetry by using methyl viologen (MV) as a mediator and by adjusting pH from the thermodynamic viewpoint. Potentiometric measurements have revealed that the three redox couples, MV2+/MV+, HCOO−/HCO3−, FoDH1 (ox/red), reach an equilibrium in the bulk solution when the two-way bioelectrocatalysis proceeds in the presence of FoDH1 and MV. The steady-state voltammograms with two-way bioelectrocatalytic properties are interpreted on a simple model by considering the solution equilibrium.

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1. Introduction

In recent years, the technology of capturing and storing renewable energy has been extensively discussed and investigated. The reduction of carbon dioxide to generate reduced carbon compounds for use as fuels and chemical feedstocks is an essential requirement for carbon-based sustainable energy economy [1]. Interconversion system of formate/carbon dioxide (HCOO−/CO2) is one of the answers for the purpose. Furthermore, this system has another merit of CO2 fixation, since CO2 is known to a major cause of the present global warming [2]. CO2 fixation helps not only to produce renewable energy and to develop new carbon cycle but also to decrease the atmospheric CO2 level [3]. Formate is the first stable intermediate during the reduction of CO2 to methanol or methane and is increasingly recognized as a new energy source [4,5]. In addition, it can easily be handled, stored, and transported. However, when CO2 is reduced and formate is oxidized directly on electrodes, a variety of products are generated and quite high overpotential is required [6,7]. The non-catalyzed thermal decomposition of formate is dominated by the reaction channels, the decarboxylation/dehydrogenation yielding carbon dioxide and hydrogen. For the desired pathway higher activation energy is necessary [8]. Catalysts developed so far to overcome this problem are inefficient and expensive [9–17]. One of the most promising strategies for solving these issues is the utilization of enzymes as catalysts. Enzymes have novel properties of substrate specificities and high catalytic efficiencies, allowing them to function in a specific biological reaction under mild conditions, such as room temperature, atmospheric pressure and neutral pH. Formate dehydrogenase (FoDH) is a key enzyme in the energy conversion reactions of methyloptrophs aerobic bacteria, fungi, and plants. The enzyme, in general, catalyzes the oxidation of formate to CO2. However, certain FoDHs have been reported to act as CO2 reductases [18–22]. It is now established that some redox enzymes are able to catalyze reactions reversibly [23]. For example, DMSO-reductase [24]; [25], CO dehydrogenase [26], fumarate:menaquinone oxidoreductase, succinate:quinone reductase [27] and some hydrogenases [28]. A great variability is found in bacterial FoDHs and they can be divided into two major classes based on their
metal content/structure and consequent catalytic strategies [29]. The metal-independent FoDH class comprises NAD-dependent FoDHs in the category of the α-specific dehydrogenases of the 2-oxyacid family [30–32]. These enzymes are found in aerobic bacteria, yeast, fungi and plants. Because these enzymes have no redox cofactors or metal ions, the formate oxidation to CO₂ has been suggested to involve the direct hydrate transfer from formate to NAD⁺. The metal-containing FoDH class comprises only prokaryotic FoDHs in the category of the molybdenum and tungsten-containing enzyme families. This class of FoDHs is composed of complex subunits with different redox cofactors, and the active site harbors one molybdenum or tungsten atom that catalyzes the proton/electron transfer in their active site, at which the formate oxidation takes place. Accordingly, the metal-containing FoDH class can be sub-divided as molybdenum-containing FoDH and tungsten-containing FoDH.

Notably, some FoDHs in the metal-containing FoDH class also comprises NAD-linked FoDH, which contains FMN to link with NAD⁺. These enzymes utilize NAD⁺ only as the electron acceptor in the biological system. These enzymes are suitable for electrochemistry because some of them can transfer electrons to electrode or artificial redox partners (mediators) [33–36]. Mediators enable the enzymatic reaction to couple with an electrode reaction by shuttling electrons between enzymes and electrodes. This reaction is called mediated electron transfer (MET)-type bioelectrocatalysis. MET-type reaction has been recognized as a key system for developing novel biosensors, bioreactors and biofuel cells, because a variety of oxidoreductase reactions can be utilized for these applications [37].

Here, we have focused on tungsten-containing formate dehydrogenases (FoDH1; EC 1.2.1.2) from Methylobacterium extorquens AM1. This enzyme is one of the NAD-linked formate dehydrogenases from this methylotroph [38]. FoDH1 is heterodimer of two identical subunits each comprising two domains (a coenzyme binding domain and a substrate binding domain) and catalyzes the oxidation of formate to CO₂ in coupled reduction of NAD⁺ to NADH [39]. It is difficult to use the NAD⁺/NADH couple as a mediator in bioelectrocatalytic system, because the electrochemical reaction of the NAD⁺/NADH couple on electrodes requires very high overpotentials. We must find other mediators such as quinines to couple the enzyme reaction with electrode reactions. FoDH1 uses ferricyanide and several oxidized dyes as electron acceptors in place of NAD⁺ [39]. Therefore, FoDH1 has possibility that it shows the CO₂ reduction activity with some suitable reduced dyes as electron donors as like as another FoDHs and can be utilized to construct a bioelectrochemical interconversion system of formate/CO₂. In this paper, we have demonstrated that FoDH1 reacts with several artificial mediators as electron donors for the reduction of CO₂ to formate as well as acceptors for the oxidation of formate to CO₂, and have evaluated the bi-molecular reaction rate constants between FoDH1 and the mediators. Furthermore, we have constructed a bioelectrochemical interconversion system between formate and CO₂ using FoDH1 and methyl viologen. The kinetics has been interpreted from the thermodynamic point of view and effects of the reversible property of FoDH1 on the catalytic current response have been detailed. Based on the thermodynamic and kinetic aspects, a strategy to get two-way bioelectrocatalytic system with one mediator has been presented.

2. Experimental

2.1. Materials

Sodium chloride, ammonium sulfate, potassium dihydrogen-phosphate, sodium formate, sodium carbonate, sodium molybdate dihydrate, 1-methoxy-5-methylphenazinium methyl sulfate (PMS), 2,6-dichlorophenolindophenol sodium salt hydrate (DCIP), 1,2-naphthoquinone (BQ), 1,4-naphthoquinone (ANQ), 2-methyl-1,4-naphthoquinone (VK₅), anthraquinone-2-sulfonic acid (AQ₂S), alizarin red S (ARS) and 1,4-benzoquinone (BQ) were purchased from Wako Pure Chemical (Japan). Benzyl viologen (BV) and sodium tungstate dihydrate were obtained from Nacalai Tesque (Japan). Methyl viologen dichloride (MV), 9, 10-phenanthrenequinone (PQ), 2,5-dichloro-1,4-benzoquinone (25DCBQ) and anthraquinone-2,7-disulfonic acid (AQ2TD) were obtained from Tokyo Chemical Industry (Japan). Hipolypepton was sourced from Nihon Seiyaku (Japan). Yeast extract and NAD⁺ were sourced from Oriental Yeast (Japan). 2,3-Dimethoxy-5-methyl-1,4-benzoquinone (Q₃₀) was obtained from Sigma–Aldrich Co. (USA). All chemicals were of analytical grade and used as received. The doubly distilled water used for sample and buffer preparation was purified with a Milli-Q water.

2.2. Purification of FoDH1

Methylobacterium extorquens AM1 (NCIMB 9133) was purchased from NCIMB (Aberdeen, Scotland, UK). The cells were grown at 28 °C in modified Luria broth, which consisted of 1% hipolypepton, 1% yeast extract, 0.5% sodium chloride, 1 μM sodium tungstate and 0.5 μM sodium molybdate. The cells were cultivated in 500 mL Erlenmeyer flasks filled with 150 mL medium. (Warning: When the cells were grown in 10-L glass fermenters containing 6 L medium, the specific activity of FoDH1 was very low (28 U mg⁻¹). Therefore, the cultures were harvested in the method described above.) The cells were collected, suspended with 20 mM potassium phosphate buffer (KPB) pH 6.0 and then disrupted two times with a French pressure cell (Otake Works, Japan) at 100 MPa. Centrifugation was performed at 100,000 × g for 1 h at 4 °C to remove cell debris. The supernatant solution was loaded on a Toyopearl DEAE-650 M column ( Tosoh Corporation, Japan) equilibrated with 20 mM KPB pH 6.0. FoDH1 was eluted with linear gradient of NaCl from 120 mM to 180 mM in the KPB pH 6.0. The sample was collected and applied to a Toyopearl Butyl-650 M column (Tosoh Corporation, Japan) equilibrated with the KPB containing 20% (w/w) ammonium sulfate. The elution of FoDH1 was carried out under a linear gradient of ammonium sulfate from 12% to 8% in the same KPB pH 6.0. All purification steps were performed at 4 °C under aerobic conditions. Protein concentrations were determined with the Pierce BCA Protein Assay Kit (Thermo Scientific, USA) using bovine serum albumin as a standard. The purities of FoDH1 were judged by Coomassie brilliant blue R-250 staining of SDS–PAGE.

2.3. Spectroscopic measurements

2.3.1. FoDH1 assays

FoDH1 activity assays were done in 1-cm light-path cuvettes with 0.1 M KPB pH 7.0. The 1-mL assay mixture contained 30 mM formate, 0.2 mM DCIP and 0.05 mM PMS. Reactions were started by the addition of the FoDH1, and the decrease in the absorbance at 600 nm due to the reduction of DCIP was measured using a Shimadzu UV-2550 UV–VIS Spectrophotometer (Japan). One unit of FoDH1 activity was defined as the amount of FoDH1 that catalyzes the reduction of 1 μmol of DCIP per min. The extinction coefficient for DCIP at 600 nm was taken as 20.6 μM⁻¹ cm⁻¹ at pH 7.0 [40]. The specific activity of the enzyme purified here was 330 U mg⁻¹.

2.3.2. The kinetic parameter of FoDH1 for NAD⁺

The kinetic parameter of FoDH1 for NAD⁺ was determined in 0.1 M KPB pH 7.0 at 30 ± 2 °C. The reaction rates were determined...
by monitoring the production of NADH at 340 nm in 1-cm light-path cuvettes by using a molar extinction coefficient of 6.22 mM\(^{-1}\) cm\(^{-1}\) [41].

2.4. Electrochemical measurements

All electrochemical measurements were carried out with an ALS 611s voltammetric analyzer in 0.1 M KPB at various pH at 30 ± 2 °C under a complete argon atmosphere. The working electrode was a glassy carbon electrode (GCE, 3 mm in diameter, BAS). The GCE was polished with 0.05-μm alumina powder, sonicated to remove it and washed with distilled water. The reference and counter electrodes were a handmade Ag/AgCl(sat.KCl) electrode and a Pt-wire, respectively. All of the potentials are referred to the reference electrode in this paper. In all of the bioelectrocatalytic experiments, the enzyme and the mediators were used in soluble (un-immobilized) forms.

3. Result and discussion

3.1. Mediated bioelectrocatalysis of formate oxidation

FoDH1 did not give a clear signal of direct electron transfer-type bioelectrocatalysis in the presence of formate (HCOO\(^{-}\)) at GCEs, as shown in Fig. S1. However, in the presence of PQ as a mediator, a large catalytic oxidation wave with sigmoidal and steady-state characteristics was observed in KPB (pH 7.0) containing 50 mM HCOO\(^{-}\), as shown in Fig. 1A (solid line). The half-wave potential (−0.18 V) is in a good agreement with the formal potential of PQ (\(E_{pQ}^0 = −0.18\) V at pH 7.0, which was evaluated as a mid-point potential in non-turnover voltammetric wave of PQ, given in Fig. 1A, broken line). The sigmoidal wave is a typical mediated bioelectrocatalysis of the HCOO\(^{-}\) oxidation. Similar bioelectrocatalytic oxidation wave was observed, when MV\(^{2+}/\)MV\(^{-}\) with \(E_{r}^{\text{MV}} = −0.63\) V was used as a mediator (Fig. 1B), but the steady-state limiting current was much smaller than that observed in the presence of PQ.

We examined the relation between the formal potential of mediator (\(E_{M}^{0}\)) and the bi-molecular reaction rate reaction constants between FoDH1 and mediators for the HCOO\(^{-}\) oxidation (\(k_{\text{cat,ox}} \equiv k_{\text{cat,ox},\text{HCOO}^{-}} / k_{\text{cat,ox},\text{red}}\) and \(k_{\text{cat,ox}}\) being the catalytic constant for the HCOO\(^{-}\) oxidation and the Michaelis constant for the oxidized form of a mediator, respectively). Under the assumptions that the FoDH1-catalyzed HCOO\(^{-}\) oxidation follows an ordinary ping-pong bi–bi mechanism, the steady-state kinetics of the HCOO\(^{-}\) oxidation reaction by FoDH1 is expressed by:

\[
\nu_{E_{\text{M,ox}}} = \frac{n_{\text{M,HCOO}^{-}}}{n_{\text{M}}} \frac{k_{\text{cat,ox,E}_{\text{M}}} c_{\text{E}}}{1 + k_{\text{HCOO}^{-}/\text{HCOO}^{2-}} c_{\text{HCOO}^{-}} + k_{\text{M,ox}} c_{\text{M,ox}}} \tag{1}
\]

When the HCOO\(^{-}\) concentration (\(c_{\text{HCOO}^{-}}\)) is much larger than the Michaelis constant for HCOO\(^{-}\) (\(K_{\text{HCOO}^{-}} = 1.6\) mM [39]), the steady-state enzymatic reduction rate of the mediator (\(i_{\text{M,red}}\)) is given by:

\[
\nu_{E_{\text{M,red}}} = \frac{n_{\text{M,HCOO}^{-}}}{n_{\text{M}}} \frac{k_{\text{cat,ox,E}_{\text{M}}} c_{\text{E}}}{1 + k_{\text{HCOO}^{-}/\text{HCOO}^{2-}} c_{\text{HCOO}^{-}} + k_{\text{M,ox}} c_{\text{M,ox}}} \tag{2}
\]

where \(n_{\text{M,HCOO}^{-}}\) (= 2) and \(n_{\text{M}}\) are the numbers of electrons of HCOO\(^{-}\) and mediator, respectively, and \(c_{\text{E}}\) and \(c_{\text{M,ox}}\) are the total concentrations of FoDH1 and the oxidized mediator in the bulk solution, respectively. The value of \(k_{\text{M,ox}}\) for FoDH1 was evaluated from the spectrophotometric monitoring of NADH during the FoDH1 reaction with NAD\(^{+}\) as an electron acceptor.

Under biocatalytic conditions of \(c_{\text{HCOO}^{-}} \gg K_{\text{HCOO}^{-}}\), the concentration polarization of HCOO\(^{-}\) becomes negligible near the electrode surface to generate steady-state sigmoidal waves, as shown in Fig. 1. When \(c_{\text{M,red}} \ll K_{\text{M,ox}}\) (\(c_{\text{M,red}}\) being the bulk concentration of the reduced mediator), the steady-state catalytic limiting current \(i_{\text{lim,ox}}\) of a mediated bioelectrocatalysis is given by [42]:

\[
i_{\text{lim,ox}} = n_{\text{M}} F A c_{\text{M,red}} \sqrt{\left(n_{\text{HCOO}^{-}} / n_{\text{M}}\right) D_{\text{M}} k_{\text{cat,ox,E}_{\text{M}}}} \tag{3}
\]

where \(F\), \(A\), and \(D_{\text{M}}\) are the Faraday constant, the electrode surface area, and the diffusion constant of a mediator, respectively. The diffusion constants were determined by hydrodynamic voltammetry for each mediator in the absence of FoDH1 and its substrate (Table S2). The \(k_{\text{M,ox}}\) values were estimated on the basis of Eq. (3) for the artificial electron acceptors that can work as the mediators.

The logarithmic values of \(k_{\text{M,ox}}\) increase linearly with \(E_{\text{M}}^{0}\) of the electron acceptors examined here up to about −0.2 V of \(E_{\text{M}}^{0}\) and reached a limiting level of \(\log(k_{\text{M,ox}}) \approx 0.85\), as shown in Fig. 2A. As pointed out in a previous paper [43], the maximum value of \(k_{\text{M,ox}}\) is in an order expected for typical diffusion-controlled reactions. In order to construct high performance biofuel cells, a large current density (that is, a rapid mediated bioelectrocatalytic reaction) is required at a potential as negative as possible. Fig. 2A indicates that PQ is the most effective mediator for the HCOO\(^{-}\) oxidation to realize a diffusion-controlled reaction with FoDH1.

The linear dependence is called a linear free energy relationship (LFE), which should be observed for reactions with no strong specific interaction, and is given by the following equation for this case.

\[
\log \left( \frac{k_{\text{M,ox,1}}}{k_{\text{M,ox,2}}} \right) = \beta_{\text{ox}} n_{\text{M}} F / 2.303RT \Delta E_{M,i} / i \tag{4}
\]
where \( \beta_{\text{ox}} \) is a proportional constant in an LFER (0 < \( \beta_{\text{ox}} < 1 \)), \( n'_{\text{ox}} \) is the number of electrons in the rate determining step, \( R \) is the gas constant, \( T \) is the absolute temperature, and \( \Delta E_{M,j} \) is the difference in the formal redox potential between mediator \( j \) and mediator \( i \) (\( E_{M,j}^{\text{ox}} - E_{M,i}^{\text{ox}} \)); \( \beta_{\text{ox}} \) is evaluated to be about 0.5 by assuming \( n'_{\text{ox}} = 1 \) from the linear dependence in the \( E_{M}^{\text{ox}} \) range from 0.7 V to 0.2 V.

It is noteworthy that the log \( k_{\text{ox}} \) value of NAD\(^+\) also locates on the linear relationship of the other artificial electron acceptors. This means that the interaction between NAD\(^+\) and FoDH1 is not so specific and NAD\(^+\) behaves as like as artificial electron acceptors, although NAD\(^+\) is regarded as the natural electron acceptor of FoDH1 in the biological system [39].

### 3.2. Mediated bioelectrocatalysis of the reduction of hydrogen carbonate reduction

As described in Section 3.1, the catalytic current of the HCOO\(^-\)/HCO\(_3^-\) couple was observed with MV in KPB of pH 7.0, as shown in Fig. 1B, solid line. It is noteworthy that the \( E_{M}^{\text{ox}} \) of MV (\( E_{M}^{\text{ox}} = -0.63 \) V) is only slightly more positive than the formal potential (\( E' \)) of the HCOO\(^-\)/HCO\(_3^-\) couple at pH 7.0 (\(-0.64 \) V [7]). The successful observation of the catalytic HCOO\(^-\) oxidation with MV at pH 7.0 has inspired expectations that FoDH1 can catalyze the reduction of hydrogen carbonate (HCO\(_3^-\)) with MV\(^+\) as an electron donor under slightly acidic conditions. Because \( E' \) of the HCOO\(^-\)/HCO\(_3^-\) couple shifts to positive direction with a decrease in pH and \( E_{M}^{\text{ox}} \) is independent of pH, the electron transfer from MV\(^+\) to HCO\(_3^-\) becomes downhill under slightly acidic conditions. In order to examine the HCO\(_3^-\) reduction, cyclic voltammetry was carried out at pH 6.6 in the presence of HCO\(_3^-\) (50 mM) and MV. When FoDH1 was added into the measurement solution, a sigmoidal and steady-state cathodic wave was observed, as shown in Fig. 1C, solid line. The wave is MV-mediated and FoDH1-catalyzed reduction of HCO\(_3^-\). We also attempted to observe the HCO\(_3^-\) reduction current with some other mediators as electron donors and evaluated the bi-molecular reaction rate constant reaction constants between FoDH1 and mediators for the HCO\(_3^-\) reduction (\( k_{\text{red}} \equiv k_{\text{cat,red}}/k_{\text{red}} \)), \( k_{\text{cat,red}} \) and \( k_{\text{red}} \) being the catalytic constant for HCO\(_3^-\) reduction and Michaelis constant for the reduced form of a mediator, respectively by:

\[
\text{lim}_{i} = -n_{\text{red}}FAC_{M,\text{ox}} \sqrt{\left( \frac{n_{\text{HCOO}}}{n_{\text{ox}}} \right) D_{M,\text{red}} C_{E}}
\]

The physical meanings of the parameters are similar to those of Eq. (3) but are related to the reduction of HCO\(_3^-\) instead of the oxidation of HCOO\(^-\). The \( k_{\text{red}} \) values also appear to follow an LFER against \( E_{M}^{\text{ox}} \).

\[
\log \left( \frac{k_{\text{red}}}{k_{\text{red}}} \right) = \frac{\beta_{\text{red}} n_{\text{red}} F}{2.303RT} \Delta E_{M,j}^{\text{ox}}
\]

The proportional constant \( \beta_{\text{red}} \) (0 < \( \beta_{\text{red}} < 1 \)) was evaluated to be about 0.5 by assuming \( n_{\text{red}} = 1 \).

### 3.3. Effects of pH on the bi-molecular reaction rate constant between FoDH1 and MV

As described in Section 3.2, \( E' \) of the HCOO\(^-\)/HCO\(_3^-\) couple is a function of pH, while \( E_{M}^{\text{ox}} \) is independent of pH. In addition, the catalytic reactions of both of the HCOO\(^-\) oxidation and the HCO\(_3^-\) reduction obey the LFER. Therefore, we can expect pH dependence of the bi-molecular reaction rate constant between FoDH1 and MV for the HCOO\(^-\) oxidation and the HCO\(_3^-\) reduction.

By considering the standard formal redox potential of the HCOO\(^-\)/HCO\(_3^-\) couple (\( E' \) (pH 7)) and \( pK_{a} \) values [35,36], \( E' \) in pH region from 6.8 to 9.0 at 30°C is given by:

\[
E'_{5} = -0.224 \, \text{V} - 2.303 \frac{m_{H}RT}{n_{\text{red}} F} \cdot \text{pH}
\]

\[
= -0.224 \, \text{V} - (0.061 \, \text{V} \times \text{pH})
\]

where \( m_{H} \) is the number of protons and \( n_{\text{red}} \) is the number of electrons in the redox reaction of the HCOO\(^-\)/HCO\(_3^-\) couple. Eq. (7) indicates a two-electron two-proton redox reaction. Therefore, by considering the LFER given by Eqs. (4) and (6), the pH dependence of the bi-molecular reaction rate constant between FoDH1 and MV can be written by:

\[
\log \left( \frac{k_{\text{red}}}{k_{\text{red}}} \right) = \beta_{\text{red}} n_{\text{red}} F \Delta pH_{j,i}
\]

for the HCOO\(^-\) oxidation and

\[
\log \left( \frac{k_{\text{red}}}{k_{\text{red}}} \right) = -\beta_{\text{red}} n_{\text{red}} F \Delta pH_{j,i}
\]

for the HCO\(_3^-\) reduction, respectively, where \( \Delta pH_{j,i} \) is the difference between \( pH_{j} \) and \( pH_{i} \).

We measured both of the anodic and cathodic catalytic currents at various pHs and evaluated \( k_{\text{ox}} \) and \( k_{\text{red}} \) values by using Eqs. (3) and (5), respectively. Fig. 3 illustrates logarithmic values of the bi-molecular rate constant against pH for (A) the HCOO\(^-\) oxidation and (B) the HCO\(_3^-\) reduction. The linear relationship was observed
between the logarithmic rate constant and pH. The straight lines in the panels have a slope expected from Eqs. (8) and (9) with \( \beta_{ox} = 0.5, n_{ox} = 1, \beta_{red} = 0.5, \) and \( n_{red} = 1. \) The results also support the LFER.

3.4. Two-way bioelectrocatalysis of HCOO\(^-\) and HCO\(_3^-\)

Based on the above results, we can safely conclude that MV can work as a two-way (both anodic and cathodic) mediator when \( \Delta G^\circ = 0 \) (that is, \( E^\circ = E^\circ_{red} \)) in the presence of both of HCO\(_3^-\) and HCOO\(^-\). Hence, we measured cyclic voltammograms in KPB (pH 7.0) containing MV, HCO\(_3^-\) (50 mM) and HCOO\(^-\) (50 mM). In the absence of FoDH1, non-turnover redox wave of MV was observed (Fig. 4 dash line). After addition of FoDH1, a steady-state sigmoidal-shaped wave appeared with both cathodic and anodic directions (Fig. 4, solid line). A similar two-way reaction has been reported for the catalytic reaction of Desulfovibrio vulgaris cells and H\(_2\) [44]. To examine the effect of pH against the two-way bioelectrocatalytic voltammogram, we recorded cyclic voltammograms at various pHs in solutions with the same composition except the buffer components. Fig. 5 shows that the steady-state current–potential curve after being corrected for the background currents at pH 6.8 ( ), pH 7.3 ( ) and pH 8.4 (■). The sigmoidal steady-state current–potential curve is shifted upward with an increase in pH.

We also performed potentiometry at several pHs in the range from 6.8 to 9.0 in the presence of FoDH1 and MV in solutions containing HCO\(_3^-\) (50 mM) and HCOO\(^-\) (50 mM). The potential reached stable values within 10 min after the addition of MV to the reaction buffer containing FoDH1 and the substrates. The equilibrated potential obtained \( E_{eq} \) is plotted against pH of the solution in Fig. 6. The straight line of the \( E_{eq} \) vs. pH plot can be expressed by a linear regression analysis as:

\[
E_{eq} = -0.056 \, V \times pH - 0.233 \, V \tag{10}
\]

This regression equation is in good agreement with the theoretical one \( (\text{Eq. (7)}) \). This implies that the electrode potential is well equilibrated by the HCO\(_3^-\)/HCOO\(^-\) couple in the solution in the presence of FoDH1 and MV, as expressed by:

\[
E_{eq} = E^\circ_{MV} + \frac{RT}{F} \ln \frac{c_{HCOO^-}}{c_{HCO_3^-}} = E^\circ_{MV} + \frac{RT}{2F} \ln \frac{c_{HCO_3^-}}{c_{HCOO^-}} \tag{11}
\]

When \( c_{HCOO^-} \ll K_{HCOO^-} \) and \( c_{HCO_3^-} \ll K_{HCO_3^-} \), the overall rate constant of the MV\(^{2+}\) reduction \( (\nu_{MV, red}) \) in the FoDH1 reaction can be written by:

\[
\nu_{MV, red} = \frac{n_{MV}}{n_{MV}} (k_{red} c_{red} c_{MV^{2+}} - k_{ox} c_{ox} c_{MV^{2+}}) \tag{12}
\]

where \( c_{ox} \) and \( c_{red} \) are the concentration of the fully oxidized and reduced form of the enzyme in the bulk solution. In the steady state, this equation can be solved by the theory of the steady-state catalytic current \( (i_s) \) of MET-type bioelectrocatalysis reaction [44] to give an equation:

\[
i_s = n_M F A c_{MV} \times \sqrt{(n_s/n_M)} D_{MV} (k_{ox} c_{ox} + k_{red} c_{red}) \left( \frac{1}{1 + K} - \frac{1}{1 + \eta} \right) \tag{13}
\]

Fig. 3. Logarithmic plots of (A) \( k_{ox} \) and (B) \( k_{red} \) against pH. The solid lines in panel A and B are LFER lines with a slope identical with Eq. (8) and Eq. (9), respectively. The error bars were evaluated by the Student t-distribution at 90% confidential level.

Fig. 4. Cyclic voltammograms of the HCOO\(^-\) oxidation and the HCO\(_3^-\) reduction by FoDH1 at \( v = 0.01 \, V \) and at a bare GC electrode in 0.1 M KPB (pH 7.0) under a complete argon atmosphere. Dash line: 44 \( \mu M \) MV + FoDH1, solid line: 44 \( \mu M \) MV + FoDH1 + 50 mM HCOO\(^-\) + 50 mM HCO\(_3^-\).

Fig. 5. Background current-corrected steady-state voltammograms of 44 \( \mu M \) MV + FoDH1 + 50 mM HCOO\(^-\) + 50 mM HCO\(_3^-\) at pH 6.8 ( ), pH 7.3 ( ) and pH 8.4 (■). The solid lines are the regression curve obtained on the basis of Eq. (13) with adequate parameters described in the text.
The ratio of $c_{\text{ox}}/c_{\text{red}}$ was calculated here from the $(k_{\text{ox}}c_{\text{red}} + k_{\text{red}}c_{\text{ox}})$ values evaluated in the non-linear regression analysis and the $k_{\text{ox}}$ and $k_{\text{red}}$ values interpolated on the lines in Fig. 3. The evaluated $E'_{\text{eq}}$ values are plotted in Fig. 6. The broken line is given by a linear regression analysis as:

$$E'_{\text{eq}} = -0.061 \times \text{pH} - 0.183 \text{ V}$$

The value of $-0.061 \text{ V}$ corresponding to the standard formal potential of the catalytic redox center of FoDH1 $(E'_{\text{eq}}(\text{pH}7.0))$ and the slope indicates a 2-electron 2-proton coupled electron transfer.

4. Conclusion

We have demonstrated that FoDH1 can utilize several artificial electron acceptors for the HCOO$^-$ oxidation. Although the characteristics of the artificial electron acceptors are entirely different from that of the native electron acceptor, NAD$, the reaction kinetics between FoDH1 and various electron acceptors including NAD$^+$ obey an LFER. This indicates that the interaction between FoDH1 and NAD$^+$ is not so specific. Therefore, the electron acceptors except NAD$^+$ can be utilized as mediators in bioelectrocatalysis of FoDH1. The present result indicates that PQ is the most effective mediator for the HCOO$^-$ oxidation to reach diffusion-controlled condition between FoDH1 and PQ. On the other hand, we have also reported that FoDH1 can catalyze the HCO$_3^-$ reduction with MV$^{\text{c/2}}$ and some other reduced dyes. When MV is utilized as a mediator, a two-way bioelectrocatalysis without overpotentials has been realized by controlling pH and the concentration of the substrates.

The three redox couples, MV$^{2+/3-}$, HCOO$^-$/HCO$_3^-$, FoDH1 (ox/red), reach an equilibrium in the bulk solution when the two-way bioelectrocatalysis proceeds in the presence of FoDH1 and MV. The voltammogram of the two-way bioelectrocatalysis has been well interpreted on a simple model of MET-type bioelectrocatalysis. The present results are very useful to construct an effective bioelectrochemical reaction for the CO$_2$ reduction and formate/oxygen biofuel cells as effective energy conversion systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sbsr.2015.07.008.

References


