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

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ABSTRACT

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 (HCO_3^-). FoDH1 catalyzes both of the HCOO oxidation and the HCO_3^- 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 HCO_3^-. The reversible reaction between $HCOO^-$ and HCO_3^- 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, $MV^{2+}/MV^{\cdot+}$, $HCOO^-/HCO_3^-$, FoDH1 (ox/red), reach an equilibrium in the bulk solution when the two-way bioelectrocatalysis proceeds in the presence of FoDH1 and MV. The steady-state voltammograms with two-way bioelectrocatalytic properties are interpreted on a simple model by considering the solution equilibrium.

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 ($HCOO^-/CO_2$) is one of the answers for the purpose. Furthermore, this system has another merit of CO_2 fixation, since CO_2 is known to a major cause of the present global warming [2]. CO_2 fixation helps not only to produce renewable energy and to develop new carbon cycle but also to decrease the atmospheric CO_2 level [3]. Formate is the first stable intermediate during the reduction of CO_2 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 CO_2 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 dec arboxylation/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 methylotrophic aerobic bacteria, fungi, and plants. The enzyme, in general, catalyzes the oxidation of formate to CO_2. However, certain FoDHs have been reported to act as CO_2 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

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metal content/structure and consequent catalytic strategies [29]. The metal-independent FoDH class comprises NAD-dependent FoDHs in the category of the D-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 CO_2 has been suggested to involve the direct hydride 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/electrons 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 CO_2 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 CO_2 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/CO_2. In this paper, we have demonstrated that FoDH1 reacts with several artificial mediators as electron donors for the reduction of CO_2 to formate as well as acceptors for the oxidation of formate to CO_2, 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 CO_2 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 dihydrogenphosphate, sodium formate, sodium carbonate, sodium molybdate dihydrate, 1-methoxy-5-methylphenazinium methyl sulfate (PMS), 2,6-dichlorophenolindophenol sodium salt hydrate (DCIP), 1,2-naphthoquinone (BNQ), 1,4-naphthoquinone (ANQ), 2-methyl-1,4-naphthoquinone (VK_3), 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 (AQ27DS) 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 (Q_0) 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 mM^{-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