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Short communication

Bioelectrocatalytic formate oxidation and carbon dioxide reduction at high current density and low overpotential with tungsten-containing formate dehydrogenase and mediators

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ABSTRACT

We show a great possibility of mediated enzymatic bioelectrocatalysis in the formate oxidation and the carbon dioxide (CO_2) reduction at high current densities and low overpotentials. Tungsten-containing formate dehydrogenase (FoDH1) from *Methylobacterium extorquens* AM1 was used as a catalyst and immobilized on a Ketjen Black-modified electrode. For the formate oxidation, a high limiting current density (j_{lim}) of ca. 24 mA cm^{-2} was realized with a half wave potential ($E_{1/2}$) of only 0.12 V more positive than the formal potential of the formate/CO_2 couple ($E^{\circ\prime}_{CO2}$) at 30 °C in the presence of methyl viologen (MV^{2+}) as a mediator, and j_{lim} reached ca. 145 mA cm^{-2} at 60 °C. Even when a viologen-functionalized polymer was co-immobilized with FoDH1 on the porous electrode, j_{lim} of ca. 30 mA cm^{-2} was attained at 60 °C with $E_{1/2} = E^{\circ\prime}_{CO2} + 0.13$ V. On the other hand, the CO_2 reduction was also realized with $j_{lim} \approx 15$ mA cm^{-2} and $E_{1/2} = E^{\circ\prime}_{CO2} - 0.04$ V at pH 6.6 and 60 °C in the presence of MV^{2+}.

1. Introduction

Electro-enzymatic devices have received considerable attention in view of clean technology to produce electricity and useful materials from renewable fuel sources. An interconversion system of the formate/carbon dioxide ($HCOO^-/CO_2$) couple is one of the promising objects for such devices (strictly speaking, CO_2 exists as hydrogen carbonate (HCO_3^-) around neutral pH, but we may simply use "CO_2" in this paper). $HCOO^-$ is an energy-rich compound and can be used as a fuel of energy conversion systems such as $HCOO^-/O_2$ biofuel cells, which are comparable to H_2/O_2 biofuel cells in terms of the theoretical standard electromotive force (ca. 1.2 V [1–3]). On the other hand, an efficient bioelectrochemical system of the CO_2 reduction may help us to produce energy-rich products or useful organic chemicals [4] and to reduce the atmospheric CO_2 level under mild conditions [5].

Recently, we have electrochemically characterized tungsten-containing formate dehydrogenase from *Methylobacterium extorquens* AM1 (FoDH1; EC 1.2.1.2) as a heterodimeric soluble enzyme [6]. It has been shown that FoDH1 produces mediated bioelectrocatalytic currents for both of the $HCOO^-$ oxidation and the CO_2 reduction. From the viewpoint of the kinetics between the enzyme and the mediators, 9,10-phenanthrenequinone (PQ) is the most effective mediator for the $HCOO^-$ oxidation. From the thermodynamic viewpoint, methyl viologen (MV^{2+}, 1,1′-dimethyl-4,4′-bipyridinium ion) is a useful mediator for the reversible catalytic reaction of FoDH1 because the midpoint potential of the $MV^{2+}/MV^{+\bullet}$ couple ($E_{m,MV}$) is very close to the formal potential of the $HCOO^-/CO_2$ couple ($E^{\circ\prime}_{CO2}$) around neutral pH. FoDH1 is expected to be applied to the construction of efficient electro-enzymatic devices utilizing the interconversion between $HCOO^-$ and CO_2.

In such bioelectrochemical devices, a large current density (j) should be realized at potentials close to $E^{\circ\prime}_{CO2}$. A promising approach to increase j is to immobilize enzymes and mediators on an electrode surface. For this purpose, Heller's group has developed osmium polymers to successfully immobilize enzymes and mediators on electrodes for a mediated electron transfer (MET)-type bioelectrocatalytic system [7]. By using this method, Tsujimura et al. recorded 100 mA cm^{-2} of limiting current density (j_{lim}) at 25 °C for a glucose oxidation [8]. However, very large overpotentials (≈ 1 V) are required in such bioelectrochemical glucose oxidation systems to get large j_{lim} because of a large formal potential difference between the substrate and the enzyme and of a large kinetic hindrance between the enzyme and the Os polymers. Development of a new mediated bioelectrocatalytic system with small overpotentials is needed.

In this paper, we attempt to construct a MET-type bioelectrocatalytic system for the $HCOO^-$ oxidation and the CO_2 reduction with high j_{lim} and very small overpotentials by using FoDH1. PQ, MV^{2+}, and a viologen-functionalized polymer (VP) were used as mediators. In

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addition, we focused on Ketjen Black (KB) as an electrode material to effectively immobilize FoDH1. The high j_{lim} of ca. 145 mA cm^{-2} was realized at 60 °C without the overpotential in practice for the $HCOO^-$ oxidation by using free MV^{2+}. Co-immobilization of FoDH1 and mediators was also attempted for practical purpose. The bioelectrochemical CO_2 reduction with high j_{lim} was also realized.

2. Experimental

2.1. Chemicals

KB was kindly donated from Lion Co. (Japan). Poly(tetrafluoroethylene) fine powder (PTFE, 6-J) was obtained from DuPont Mitsui Fluorochemicals (Japan). MV^{2+} dichloride and PQ were obtained from Tokyo Chemical Industry (Japan). Poly(vinylpyrrolidone) (PVP) and poly(ethylene glycol) diglycidyl ether (PEGDGE) were obtained from Sigma-Aldrich Co. (USA). Other chemicals were obtained from Wako Pure Chemical (Japan). FoDH1 was purified according to the literature [6].

2.2. Preparation of FoDH1-modified electrodes

KB slurry (KB:PTFE = 8:2 (*w*/*w*) in 2-propanol) was applied on a glassy carbon electrode (GCE) for rotating disk voltammetry, and a KB-modified GCE (KB/GCE) was prepared according to the literature [9]. The projective surface area of the GCE was 0.071 cm^2. FoDH1 was immobilized on the KB/GCE by immersing the electrode in 40 μL of an FoDH1 solution (50 μM of FoDH1 solution in 100 mM potassium phosphate buffer (KPB) of pH 6) containing 0.5% (*v*/*v*) glutaraldehyde for 24 h at 4 °C. The bioelectrode is called FoDH1/KB/GCE.

2.3. Preparation of FoDH1/PQ co-immobilized KB/GCEs

A PQ-modified KB/GCE (PQ/KB/GCE) was prepared by immersing the KB/GCE in DMSO containing 100 mM of PQ for 12 h at room temperature. After washing the electrode with 100 mM KPB (pH 7.0), FoDH1 was immobilized on the PQ/KB/GCE in a manner similar to that described in Section 2.2. The bioelectrode is called FoDH1/PQ/KB/GCE.

2.4. Preparation of VP and FoDH1/VP co-immobilized KB/GCEs

N-(4-bromobutyl)-*N*′-methyl-4,4′-bipyridinium dibromide was synthesized according to the literature [10]. VP was prepared by dissolving *N*-(4-bromobutyl)-*N*′-methyl-4,4′-bipyridinium dibromide (1.1 g, 3 mmol) and PVP (0.5 g, 10 μmol) in 100 mL of DMF, and the solution was stirred for 4 d at 45 °C. The product (VP) was precipitated in diethyl ether and dried. 4 μL of 100 mM KPB (pH 7.0) reaction solution containing 90 mg mL^{-1} VP, 20 mg mL^{-1} PEGDGE, and 18 mg mL^{-1} FoDH1 was cast onto the surface of the KB/GCE. The electrode was dried at 4 °C for 3 h. The bioelectrode is called FoDH1/VP/KB/GCE.

2.5. Electrochemical measurements

All electrochemical measurements were carried out in 1.0 M KPB at various pHs and at various temperatures under a complete argon atmosphere on an electrochemical analyzer BAS CV-50W. The working electrodes were rotated with a RDE-1 (BAS, USA). A homemade Ag/AgCl/sat.KCl electrode and a Pt-wire were used as the reference electrode and the counter electrode, respectively. All of the potentials are referred to the reference electrode in this paper.

3. Results and discussion

3.1. $HCOO^-$ oxidation at FoDH1-modified KB/GCEs

Fig. 1A shows rotating disk cyclic voltammograms (RDVs) at FoDH1/KB/GCEs in the presence of $HCOO^-$ and MV^{2+}. The $E_{m,MV}$ was −0.63 V. The sigmoidal curves represent the catalytic oxidation of $HCOO^-$, in which FoDH1 and MV^{2+} work as a catalyst and a mediator, respectively. The catalytic current completely disappeared by HCl-treatment of the bioanode. The current reached the limiting value at potentials more positive than −0.45 V and the half-wave potential ($E_{1/2}$) was −0.58 V, which is only 0.12 V more positive than $E^{\circ\prime}_{CO2}$ (−0.70 V at pH 8.0 [11]). The j_{lim} value increased with the bulk concentration of MV^{2+} (c_{MV}) in a manner of Michaelis Menten-type saturation curve (Fig. 1A, inset). The apparent Michaelis constant against MV^{2+} was estimated to be 1.0 ± 0.2 mM, the error being an asymptotic standard one in non-linear regression. The j_{lim} value was almost independent of the rotation rate (ω) at $\omega > 600$ rpm (Fig. 1B), indicating that j_{lim} is predominantly governed by the enzymatic kinetics. Under the conditions, j_{lim} of the $HCOO^-$ oxidation reached 24 ± 3 mA cm^{-2} at 30 °C and at $\omega = 1000$ rpm ($\sqrt{\omega} = 10.23\ s^{-1/2}$).

When the solution temperature was increased up to 60 °C to improve the enzymatic kinetics, j_{lim} showed a clear dependence on ω and was enhanced to 145 ± 6 mA cm^{-2} at $\omega = 6000$ rpm (Fig. 1C). This is the highest j_{lim} reported so far for the $HCOO^-$ oxidation at enzymatic bioanodes. The inset in Fig. 1C shows the Koutecký–Levich plot based on: [12]

$$\frac{1}{j_{lim}} = \frac{1}{j_D} + \frac{1}{j_{cat}} \quad (1)$$

where j_D is the Levich-type diffusion-controlled current density and j_{cat} is the enzymatic kinetics-controlled current density. The non-linear regression analysis by Gnuplot® with Eq. (1) provided a result that $j_{cat} = 294 \pm 52$ mA cm^{-2} and $j_D/\omega^{1/2} = 11 \pm 1$ mA $s^{1/2}$ cm^{-2}. The large value of j_{cat} indicates that this FoDH1-based system is very useful for the $HCOO^-$ oxidation.

Since PQ is a better electron acceptor than the natural one (NAD^+) from the kinetic viewpoint [6], we tried to use PQ as a mediator and prepared FoDH1/PQ/KB/GCEs to construct a useful bioelectrocatalytic system for the $HCOO^-$ oxidation. PQ adsorbed on a KB/GCE in a manner of Langmuir isotherm (data not shown). The absorbed PQ gave a pair of redox peaks (Fig. 2; b0) at $E_m = -0.20$ V. The electrodes produced large anodic currents of the catalytic oxidation of $HCOO^-$ (Fig. 2; b1, b2). The j_{lim} value was almost independent of ω at 30 °C, indicating the characteristics predominantly controlled by the enzymatic kinetics. The j_{lim} that increased up to ca. 35 mA cm^{-2} at 60 °C, however, is much smaller than that expected from the bimolecular rate constant (which is 1000 times larger than that of MV^{2+} [6]), in spite of a rather large overpotential. Most probably, the restricted movement of the adsorbed PQ interferes with the enzymatic reaction between the adsorbed PQ and the immobilized FoDH1.

Although the MV^{2+}-system shows very small overpotential, MV^{2+} is very soluble and it is difficult to immobilize MV^{2+} on electrodes. Therefore, we synthesized VP and immobilized it on KB/GCEs. The $E_{m,VP}$ was −0.52 V (Fig. 2; a0) and slightly more positive than $E_{m,MV}$, probably due to the repulsion between the positive charges in the oxidized polymer. In the presence of $HCOO^-$, the FoDH1/VP/KB/GCEs produced large anodic *j* (Fig. 2; a1, a2) comparable with that of FoDH1/PQ/KB/GCEs. The j_{lim} value reached ca. 30 mA cm^{-2} at 60 °C and at $\omega =$ 1000 rpm. The $E_{1/2}$ was −0.57 V and only 0.13 V more positive than $E^{\circ\prime}_{CO2}$. These results suggest that the VP-immobilized system can well mediate the electron transfer between the immobilized FoDH1 and the KB/GCE compared with the PQ-adsorbed system. Such a polymer system seems to retain some extent of the mobility, which is essential in MET-type bioelectrocatalysis. The flexibility seems to increase at