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Electrochemical Conversion of Carbon Dioxide to Methanol with Use of Enzymes as Biocatalysts

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Electrolysis of carbon dioxide-saturated phosphate buffer solution (pH 7) in the presence of formate dehydrogenase and methanol dehydrogenase as electrocatalysts together with methylviologen as an electron relay yielded formaldehyde and methanol as the reduction products of carbon dioxide under suitable electrolysis conditions, and the use of pyrrolo-quinoline quinone in place of methylviologen as the electron relay afforded selective reduction of carbon dioxide to methanol.

Electrochemical reduction of carbon dioxide to liquid fuels such as methanol using water as a proton source has been a challenging subject. The reduction at metal electrodes usually yields two electron reduction products of formic acid and carbon monoxide depending on the kind of metals used,1,2) except for copper where methane and ethylene are produced under appropriate electrolysis conditions.3) The reduction in the presence of metal complexes as electrocatalysts such as nickel tetraaza macrocycles,4) metal phtalocyanine and porphyrin complexes,5) and polypyridyl metal complexes6) also yield the two electron reduction products. Although the achievement of reduction to methanol has been reported for the use of GaAs,7,8) Ru,9) and RuO_2-$TiO_2$10) as the cathode materials, GaAs is apt to be cathodically decomposed,11) and RuO_2-TiO_2 also seems to have the same tendency more or less. As for Ru predominant evolution of hydrogen is expected to occur due to low hydrogen overvoltage of this material.12) Only one promising approach for direct reduction of carbon dioxide to methanol with high selectivity might be electrolysis of carbon dioxide-saturated aqueous electrolyte solutions at potentials very close to the thermodynamic potential for reduction to methanol,13) where no appreciable hydrogen evolution occurs. In this communication, a novel electrochemical means for reduction of carbon dioxide to methanol is reported, where two kinds of enzymes of formate dehydrogenase and methanol dehydrogenase are used as the electrocatalysts. To our best knowledge, no report has been published on the achievement of electrochemical production of methanol from carbon dioxide with assistance of enzymes as the electrocatalysts.

Formate dehydrogenase (FDH) is an enzyme that catalyzes oxidation of formate to carbon dioxide in bacteria. The reverse reaction, i.e., reduction of carbon dioxide to formate, can be induced to occur electrochemically with the use of FDH as an electrocatalyst in the presence of methylviologen (MV^{2+}) as an electron relay.14,15) Methanol dehydrogenase (MDH) catalyzes oxidation of methanol in bacteria with cytochrome C as an oxidizing agent. MDH (EC 1,1,99,8) contains pyrroloquinoline quinone (PQQ) as a cofactor, and shows activities for oxidation of not only n–aliphatic alcohols but also formaldehyde to formic acid.[16)] As in the case of FDH, it may be expected to induce elecrochemically the reverse reaction, i.e., reduction of formate to methanol, but no report has been published for the capability of this enzyme for the reverse reaction. As will be shown below, MDH was found to work as an electrocatalyst for reduction of formate to methanol or to both methanol and formaldehyde, depending on the kind of the electron relays used. Based on this discovery, a novel electrochemical reduction route of carbon dioxide to methanol utilizing concerted works of FDH and MDH has been devised.

FDH (EC 1,2,1,2) and MDH (EC 1,1,99,8) were purchased from Sigma. The former was from Pseudomonas oxalaticus, and the latter from Methylophilus methylotrophus. It was essential to use an electron mediator to electrically communicate the enzyme with the electrode. Methylviologen that works as a useful electron relay for FDH was used for this purpose. In addition, capabilities of the electron mediation of PQQ, which is a cofactor of MDH, were examined. All the electrolysis experiments were carried out in the dark using a two compartment cell separated by a cation exchange membrane (Nafion 117). The capacity of the cathode compartment was 5 cm^3 and a glassy carbon plate of 1 cm^2 area was used as a test electrode. A phosphate buffer solution of pH 7 containing 0.3 mol dm^{-3} $NaHCO_3$ was used as an electrolyte solution. The electrolysis was carried out under magnetically stirring the electrolyte solutuions. In the beginning of the electrolysis at a fixed cathodic potential, relatively large currents of several hundreds microampere flowed due to reduction of the electron relay used, but soon decayed to steady currents of several tens microampere, after which the reduction of carbon dioxide or sodium formate used as the starting material mostly proceeded.

The amount of formate produced was determined by high performance liquid chromatography, that of formaldehyde by a colorimetric method using chromotropic acid, and that of methanol by gas chromatography. The current efficiency (η) for a reduction product was determined by Eq. 1,

$$\eta = (\text{the number of moles of product}) \times (n)/[Q_A - Q_B] \times 96500 \qquad (1)$$

where n is the number of elecrons involved in reduction of a reactant to the product, and Q_A and Q_B are the total electrolysis charge employed and the charge that is theoretically required to reduce all mediators used in the electrolysis experiments, respectively. The turnover number of FDH was determined with the assumption that the activity of the enzyme was 7.2×10^3 unit g^{-1} and its molecular mass was 315000.[17)] In the case of MDH, an activity of 1.7×10^3 unit g^{-1} and the molecular mass of 128000 were assumed for methanol production.[16)]

The electrolysis at –0.8 V vs. SCE of carbon dioxide–saturated phosphate buffer solution in the presence of FDH and MV^{2+} yielded formaldehyde, as expected. It was found that PQQ worked well as an electron relay for FDH, though the production rate of formate was low compared to the use of MV^{2+}. Typical electrolysis results are given in Table 1. The amount of formate produced was influenced by the amount of FDH and MV^{2+}. The low production rate observed at PQQ does not neccesarily mean low current efficiencies for formate production. The curent efficiency of 89%, which is comparable to the current efficiency obtained with the use of MV^{2+}, was obatined. The low production rate was entirely indebted to small electrolysis currents under controlled potential electrolysis at –0.8 V vs. SCE. The regeneration of the enzyme by PQQ must be slow compared to that by MV^{2+}. The turnover number of FDH achieved at the electrolysis for 20 h was 6300 for the use of MV^{2+} and 890 for the use of PQQ.

Electrolysis at –0.7 V vs. SCE for 20 h of 10 mmol dm^{-3} sodium formate in the phosphate buffer solution under nitrogen atmosphere resulted in both formaldehyde and methanol when 12.5 unit MDH for 5 cm^3 solution and 5 mmol dm^{-3} MV^{2+} were used. As the time course given in Fig. 1 shows, formate is first reduced to formaldehyde, and when formaldehyde accumulates the methanol production begins. As shown in table 1, the current efficiency obtained in that case for formaldehyde production was 39 % and that for methanol 45%. The turnover number of the enzyme for methanol production was 19.1 at that stage. It was found that if the amount of MDH was decreased, for example, to 2.5 unit a small amount of formaldehyde only (0.32 μmol) was produced, and no methanol production was observed. It has been reported that MDH shows high activities in solutions of pH 9 in the presence of ammonium cations.[18] However, changes of solution pH and addition of NH_4^+ to the sodium phosphate solution did not result in any appreciable change in the reduction behaviors of formate. Interestingly, when PQQ was used as the electon relay, methanol alone was obtained as the reduction product of formate, as shown in Fig. 1 and Table 1. In that case, a smaller amount of 2.5 unit of MDH seemed enough to give high current efficiency of 92.0% for methanol production. The turnover number of MDH in that case was 95.5. Presently it is not known why PQQ gives methanol, while MV^{2+} mainly formaldehyde. Presumably interaction of the enzyme with the electron relays would be defferent between the two kinds of electron relays.

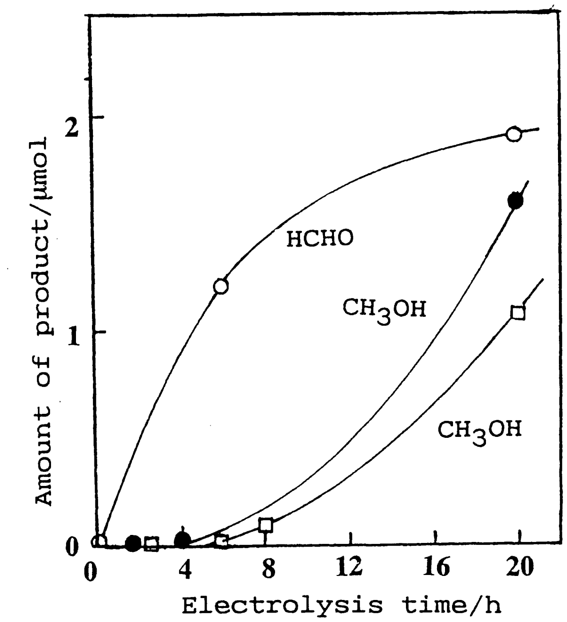

Fig. 1. Electrochemical reduction of 10 mM sodium formate in phosphate buffer containing (○,●) 5 mM MV^{2+} and 12.5 unit MDH, and (□) 1 mM PQQ and 2.5 unit MDH. E = −0.8 V vs. SCE.

Table 1. Electrochemical Reduction of Carbon Dioxide and Sodium Formate

Substance	Enzyme/Unit	Electron relay	E (V vs.SCE)	Time[a)] (h)	Product/amount (μmol)	η[b)]
CO_2	FDH / 2.5	5 mM MV^{2+}	−0.8	20	$HCOO^-$ / 7.0	90
CO_2	FDH / 2.5	5 mM PQQ	−0.8	20	$HCOO^-$ / 1.04	89
10 mM[c)] formate	MDH / 2.5	5 mM MV^{2+}	−0.7	20	HCHO / 0.32	90
10 mM formate	MDH /12.5	5 mM MV^{2+}	−0.7	20	HCHO /1.9	39
					CH_3OH / 1.1	45
10 mM formate	MDH / 2.5	5 mM PQQ	−0.7	20	CH_3OH / 1.4	92
CO_2	FDH/2.5+MDH/2.5	1 mM PQQ	−0.8	70	CH_3OH / 0.6	88
CO_2	FDH/2.5+MDH/2.5	5 mM PQQ	−0.8	70	CH_3OH / 0.9	90

a) Electrolysis time. b) Current efficiency for products. c) M = mol dm^{-3}.

The results obtained in the use of PQQ as the electron relay show that carbon dioxide is reduced to formate with assistance of FDH and that formate is reduced to methanol with assistance of MDH. Accordingly it is expected that if electrolysis of carbon dioxide-saturated phosphate buffer solutions is attempted with use of both FDH and ADH in the presence of PQQ, methanol is produced with concerted works of the two kinds of enzymes. This speculation was confirmed experimentally as shown in Table 1. The current efficiency given in this table were reproducible with errors of few percents. The concentration of PQQ more than 1 mM seemed necessary to get high current efficiencies for methanol production. Interestingly, no formate was produced in two different experiments, the reason for this being unknown at present, however. Experiments to clarify many unbiguous points are currently under way.

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