Electrochemical Conversion of Carbon Dioxide to Methanol with the Assistance of Formate Dehydrogenase and Methanol Dehydrogenase as Biocatalysts

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Abstract: Electrolysis at potentials between –0.7 and –0.9 V *vs* SCE of carbon dioxide-saturated phosphate buffer solutions (pH 7) containing formate dehydrogenase (FDH) and either methyl viologen (MV^{2+}) or pyrroloquinolinequinone (PQQ) as an electron mediator yielded formate with current efficiencies as high as 90%. The enzyme was durable as long as the electrolysis was carried out in the dark. Electrolysis of phosphate buffer solutions containing sodium formate in the presence of methanol dehydrogenase (MDH) and MV^{2+} at –0.7 V *vs* SCE yielded formaldehyde if the concentration of the enzyme used was low, whereas both formaldehyde and methanol were produced for relatively high concentrations of the enzyme where the methanol production began to occur when the formaldehyde produced accumulated. The use of PQQ in place of MV^{2+} as the electron mediator exclusively produced methanol alone after some induction period in the electrolysis. On the basis of these results, successful attempts have been made to reduce carbon dioxide to methanol with cooperative assistance of FDH and MDH in the presence of PQQ as the electron mediator. The role of enzyme and mediator in these reduction processes is discussed in detail.

Introduction

Electrochemical reduction of carbon dioxide has been fairly intensively studied. The reduction of carbon dioxide at metal electrodes in aqueous solutions usually yields formate and/or carbon monoxide, depending on the kind of metals used,[1] except for a copper electrode where methane and ethylene are produced under appropriate electrolysis conditions.[2] The use of metallocomplexes such as metal cyclams,[3] metal–phthalocyanines,[4] and metal–bipyridine complexes[5] also gives either carbon monoxide or formate as a major product. The electrochemical reduction of carbon dioxide to methanol is thermodynamically possible, but there seems to be no well-established technique to achieve this reaction with high current efficiencies close to 100%, though the methanol production has been reported with the use of ruthenium,[6] gallium arsenide,[7] and RuO_2–TiO_2 mixed cathodes.[8] The use of these electrodes usually causes appreciable amounts of hydrogen evolution as the side reaction of the methanol synthesis. Furthermore, instabilities of the cathodes are expected more or less for the use of GaAs[9] and RuO_2–TiO_2 mixed cathodes in aqueous solutions. In order to conquer these serious drawbacks, it is of significance to examine the possibility of other approaches. This paper describes the usefulness of enzymes for the electrochemical reduction of carbon dioxide to methanol.

The utility of enzymes in electroreductive fixation of carbon dioxide in organic molecules has been demonstrated in photochemical fixation to yield isocitrate in α-oxoglutaric acid and malate in pyruvic acid.[10] These enzymatic fixation reactions can also be carried out in electrochemical reaction systems[11] where the current efficiencies approaching 100% can be achieved at potentials more positive than those reported so far for electrochemical reduction of carbon dioxide at metal electrodes with and without the use of metallocomplexes as the electrocatalysts.

Our strategy for the electrochemical reduction of carbon dioxide to methanol is to use two kinds of enzymes of formate dehydrogenase (FDH) and methanol dehydrogenase (MDH). FDH (E.C. 1.2.1.2) is an enzyme that catalyzes oxidation of formate

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to carbon dioxide in bacteria with the assistance of nicotinamide adenine dinucleotide (NAD^+),[12] and it is well-known that it works as a biocatalyst for the reverse reaction of the *in-vivo* oxidation of formate, *i.e.*, for reduction of carbon dioxide to formate with the assistance of reducing agents such as NADH[13] and methyl viologen cation radial ($MV^{\bullet+}$).[10b,14] MDH is an enzyme that catalyzes oxidation of primary alcohols, such as methanol and ethanol. There are several kinds of alcohol dehydrogenase, but some of them such as ALDH (E.C. 1.1.1.1) and ALDH (E.C. 1.1.1.2) can oxidize only alcohols to yield the corresponding aldehydes. The MDH (E.C. 1.1.99.8) used in this study can, however, catalyze oxidation of not only methanol to formaldehyde but also formaldehyde to formate.[15,16] Although to the author's knowledge no report has been published concerning the achievement of the reverse reaction using this enzyme as a biocatalyst, this enzyme has the possibility of catalyzing reduction of formate to methanol *via* formaldehyde, as reported in our recent communication.[17] Then it is possible to electrochemically synthesize methanol from carbon dioxide with utilization of the cooperative works of FDH and MDH in the presence of electron mediators. In the present paper, results obtained by electrolyses using each enzyme and those using both enzymes together will be described in detail to achieve electrochemical reduction of carbon dioxide to methanol with high current yields, and discussion will be given focusing on the role of enzymes and mediators in the reduction processes. The electron mediators used were methyl viologen (MV^{2+}) and pyrroloquinolinequinone (PQQ). It has been shown already that MV^{2+} works as the mediator for FDH,[10b,14] and PQQ is a coenzyme for MDH (E.C. 1.1.99.8).[18] As will be shown, the reduction behavior of carbon dioxide to methanol is quite different for these two types of electron mediators.

Experimental Section

FDH (E.C. 1.2.1.2) obtained from *Pseudomonas oxalaticus* and MDH (E.C. 1.1.99.8) obtained from *Methylophilus methylotophus* were commercially available from Sigma. PQQ was manufactured by UBE industries. The other chemicals used were of reagent grade from Wako Pure Chemicals Co., Ltd. Methanol used as a standard for gas chromatography was distilled after being stored in the presence of solid sodium. Water was purified by two distillations with deionized water. Electrolysis experiments were carried out using a CO_2-saturated phosphate buffer solution having pH 7 prepared by mixing a 0.5 mol dm^{-3} H_3PO_4 solution and a 0.5 mol dm^{-3} KH_2PO_4 solution, both of which contained 0.3 mol dm^{-3} $NaHCO_3$, followed by bubbling CO_2 gas for more than 1 h. When CO_2-free phosphate buffer solution was required, the above two kinds of phosphate solutions excluding $NaHCO_3$ were mixed so as to give pH 7, and the resulting solution was saturated with N_2.

The electrode used was a glassy carbon (Tokai Denkyoku) having an exposed area of 1 cm^2. Prior to the measurements the electrode surface was successively polished with No. 2000 emery papers, 1.0 and 0.3 μm alumina. The mirror-finished electrode prepared in this way was subjected to ultrasonication in distilled water to get rid of adsorbed alumina particles. A platinum foil having a 5-cm^2 area and a saturated calomel electrode (SCE) served as a counter electrode and a reference electrode, respectively. A Hokuto Denko Model HA-301 potentiostat, a Nikko Keisoku Model HPS-2A potential sweeper, a Hokuto Denko Model HF-202D electronic coulometer, and a Yokogawa Electric Model 3078 X–Y recorder were used in the electrochemical measurements.

The electrolysis cell used was an H-type two-compartment cell in which test and counter electrode compartments were separated by a cation exchange membrane (Nafion 117). The electrolyte solution to be examined was put into the test electrode compartment, while the counter electrode compartment was filled with the same phosphate buffer solution that contained neither enzyme nor electron mediator. The top of the test electrode and counter electrode compartments were tightly fitted with silicon rubber stoppers. The stopper of the test electrode compartment had a sealed glass tube which mounted an electrical lead wire of a glassy carbon test electrode, a saturated calomel reference electrode, and a short glass tube sealed with a rubber septum (Aldrich) at one end, through which a stainless steel tube of 0.5 mm inner diameter was bridged to a graduated glass tube (20 cm height and 5 mm inner diameter) through a sealed rubber septum at its top. The open end of the graduated glass tube was immersed in distilled water in a conical flask during electrolysis experiments, and the water level in the graduated glass tube was monitored to confirm air-tightness of the compartment. When CO_2 or N_2 was bubbled into electrolyte solutions before the electrolysis experiments were carried out, the stainless steel tube penetrating through the rubber septum capped on the top end of the short glass tube was pushed down into the electrolyte solution, and the gas was introduced from the bottom end of the graduated glass tube for 1 h. Then the stainless steel tube was pulled up above the electrolyte solution, and simultaneously the bottom end of the graduated glass was immersed in distilled water. The stopper of the counter electrode compartment had a sealed glass tube which mounted a lead wire of a Pt test electrode and the same air-tightness monitor apparatus as that described above for the test electrode compartment. Prior to electrolysis experiments, the dissolved oxygen in the electrolyte solution was purged off by bubbling N_2 with the same procedure as given above for the test electrode compartment. All electrolysis experiments were carried out in the dark under magnetic stirring except where specially noted.

The determination of formate was carried out by using a Tosoh high-performance liquid chromatography system composed of a CCPK pump, an 8011 UV detector, and an organic acid column (Waters). To determine formate in solutions containing PQQ, a Tosoh ODS-120 column was connected in series to the organic acid column to separate formate from PQQ. The elute used was a 0.9% H_3PO_4 solution prepared using twice-distilled water, and its flow rate was 0.5 mL min^{-1}. The column temperature chosen was 60 °C. Formaldehyde was colorimetrically determined using chromotropic acid,[19] and methanol by gas chromatography using a Yanaco Model G180 equipped with a FID detector and Porapak T column at 100 °C. Argon gas at 3.0 kg cm^{-2} was used as a carrier gas.

When the electrolysis was initiated, large electrolysis currents whose magnitudes depended on the concentration of the electron mediator were observed but decayed to an almost constant value that depended on the electrochemical reduction rate of the respective substrates. The magnitude of the current used with 5 mM MV^{2+}, for example, was initially *ca.* 0.7 mA cm^{-2}, and steady currents less than 60 μA cm^{-2} were obtained by electrolysis for 2 h. These results indicate that the mediator was preferentially reduced in the initial stage of electrolysis. Then the net current efficiency (η) for the reduction of substrates was evaluated on the basis of eq 1

$$\eta = \frac{\text{moles of product} \times n \times 96500}{Q_A - Q_B} \quad (1)$$

where n is the number of electrons involved in the reaction for each product, and Q_A and Q_B denote respectively the quantity of electrical charge used in the electrolysis and that theoretically required for complete reduction of the electron mediators used in the electrolysis experiments. The turnover number of FDH and MDH was evaluated on the basis of the molar ratio of the product to the enzyme used. The number of moles of FDH used was evaluated by assuming that its molecular mass is 315 000 and its specific activity is 7170 units g^{-1}.[20] The turnover number of MDH was determined in a similar way with the assumption that its molecular mass is 128 000 and its specific activity is 1700 units g^{-1}.[16]

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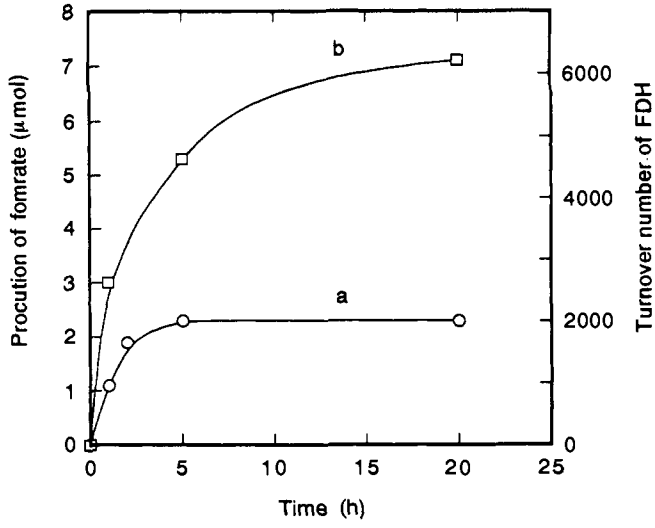

Figure 1. Time course of formate production by electrochemical reduction at –0.8 V *vs* SCE of 5 mL of CO_2-saturated phosphate buffer solution containing 2.5 units of FDH, 5 mmol dm^{-3} MV^{2+}, and 0.3 mol dm^{-3} $NaHCO_3$ without (a) and with (b) light shielding of the electrolysis cell.

Results and Discussion

Electrochemical Reduction of Carbon Dioxide to Formate with FDH. Parkinson and Weaver have achieved electrochemical reduction of carbon dioxide to formate by enforcing the reverse reaction of *in-vivo* oxidation of formate catalyzed by FDH with the use of MV^{2+} as an electron mediator.[14] However, their results showed that FDH is not durable and its activity decreases with promotion of the electrolysis. This was found to be true in our experiment if the electrolysis experiments were carried out without shielding the electrolysis cell from visible lights. As the time course of the formate production given by curve a of Figure 1 shows, complete loss of the enzymatic activity was observed for the initial 5-h electrolysis without shielding at –0.8 V *vs* SCE of CO_2-saturated phosphate buffer solution containing 2.5 units of FDH and 5 mmol dm^{-3} MV^{2+}. In that case, electrolysis currents of *ca.* 0.7 mA cm^{-2} were observed at the initial stage but decreased with the electrolysis for 5 h to less than 0.1 μA cm^{-2}, which was regarded as residual currents. In contrast, the light shielding of the cell allowed the succeeding electrolysis with currents of *ca.* 20 μA cm^{-2}, and formate was produced by the time course given by curve b of the same figure, indicating that FDH kept its enzymatic activity in the dark. This finding is in conformity with the finding reported by Höpner and Trautwein[21] that FDH is apt to lose its activity with exposure to visible lights. All the following experiments were then carried out in the dark.

The turnover number of the enzyme obtained exceeded 6000 and the current efficiency was as high as 90% for the 20-h electrolysis. The current efficiencies obtained under several different conditions were also about 90% as shown in Table 1. Exhaustive analyses of the electrolyte solution and the gas phase in the test electrode compartment revealed that formate was the sole product of CO_2 reduction. Then the fate of the rest of the charges is a matter for discussion. We speculate as the most likely cause that oxygen produced in the counter electrode compartment as a result of oxidation of water entered the test electrode compartment through the Nafion membrane and caused oxidation of $MV^{\bullet+}$ to MV^{2+}, resulting in a decrease in the current efficiency as observed. A saturation tendency in the formate production as shown by curve b of Figure 1 must result from gradual predominance of the spontaneous enzymatic reaction of oxidation of formate.

As shown in Table 1, an increase in the amount of both MV^{2+} and FDH in the electrolyte solution was effective to enhance the formate production. Changes in the cathode potential from –0.8 to –0.7 V *vs* SCE did not result in any remarkable differences in the reduction behaviors of carbon dioxide to formate, but the electrolysis at –0.9 V *vs* SCE caused a small decrease in the formate production and its current efficiency. Judging from the voltammetric behavior of MV^{2+}, electrolysis at –0.9 V *vs* SCE must reduce a very small amount of $MV^{\bullet+}$ to MV^0, which may have unfavorable influences on the electrolysis system, such as decomposition of enzyme.

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Table 1. Electrochemical Reduction of Carbon Dioxide to Formate[a] Catalyzed by Formate Dehydrogenase with the Use of MV^{2+} as an Electron Mediator

concn of MV^{2+} (mmol dm^{-3})	amount of FDH (unit)	*E* vs SCE (V)	amount of formate (μmol)	current eff (%)
1	0.25	–0.8	0.49	91
5	0.25	–0.8	0.89	90
5	0.50	–0.8	2.1	89
5	2.50	–0.8	7.2	90
5	2.50	–0.7	7.3	91
5	2.50	–0.9	5.8	79

[a] The electrolyte solution used was 5 mL of CO_2-saturated phosphate buffer (pH 7) containing 0.3 mol dm^{-3} $NaHCO_3$. The electrolysis was performed for 20 h using a glassy carbon electrode having 1 cm^2 area.

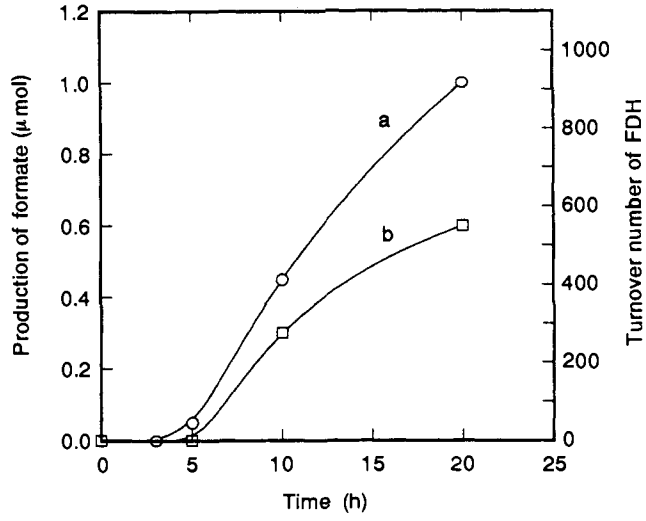

Figure 2. Time course of formate production by electrochemical reduction at –0.8 V *vs* SCE of 5 mL of CO_2-saturated phosphate buffer solution containing 2.5 units of FDH, 0.3 mol dm^{-3} $NaHCO_3$, and 5 (a) or 1 (b) mmol dm^{-3} PQQ.

As shown in Figure 2, PQQ can also work as an electron mediator for FDH in the reduction of carbon dioxide to formate. However, as noticed in this figure there appeared an induction period of several hours before the formate production began to occur and the rate of formate production was very low compared to the case with MV^{2+}. Furthermore, the rate of the formate production seems not to be simply proportional to the concentration of PQQ. These findings may be related to adsorption of PQQ on the electrode as will be described in the next section. The usefulness of PQQ as the electron mediator is not predicted straightforwardly, because the redox potential of PQQ in aqueous solution of pH 7 is –0.175 V *vs* SCE,[22] while the thermodynamic potential of the reduction of carbon dioxide to formate is –0.66 V *vs* SCE at the same pH.[23] Accordingly, PQQ originally has no inclination for electron mediation for FDH in the reduction of carbon dioxide, although the formate production did occur. It

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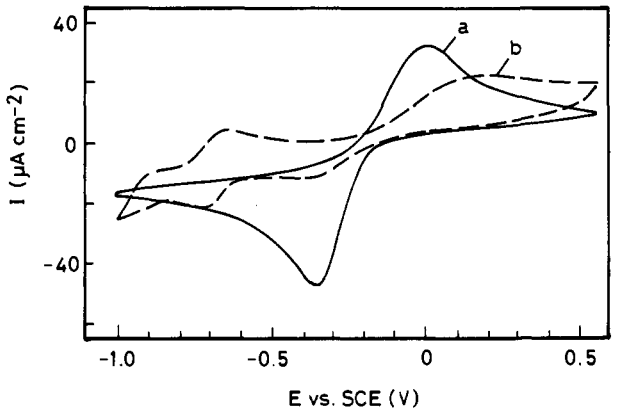

Figure 3. Cyclic voltammograms of 5 mL of CO_2-saturated phosphate buffer solution containing 2.5 units of FDH, 1 mmol dm^{-3} PQQ, and 0.3 mol dm^{-3} $NaHCO_3$ taken before (a) and after (b) electrolysis at –0.8 V *vs* SCE for 20 h. dE/dt = 5 mV s^{-1}.

was discovered by cyclic voltammetric measurements before and after the potentiostatic electrolysis at –0.8 V *vs* SCE for 20 h that the reducing power of PQQ was greatly enhanced during the course of electrolysis. As shown in Figure 3, a couple of original redox waves were greatly suppressed by the electrolysis for 20 h, and simultaneously a couple of new redox waves appeared at around –0.7 V *vs* SCE. As will be mentioned in the next section, the new redox waves seem to result from electrochemical responses of species originating from PQQ molecules which were adsorbed on the glassy carbon electrode during the course of electrolysis. This species is tentatively denoted here as adsorbed PQQ. The appearance of the induction period in the formate production can be related to slow formation of the adsorbed PQQ.

Considering that the amount of the adsorbed PQQ is by far less than that of free PQQ in the solution, the voltammetric response of the latter should appear, being in marked contrast to the experimentally obtained results. The adsorbed PQQ must block the electron exchange between free PQQ and the electrode. This hypothesis is also supported by the finding that the adsorbed PQQ blocks the redox reaction of MV^{2+} (see the last section). Since the adsorbed PQQ molecules work as the electron mediator for FDH, they must be very close to the redox center of FDH and behave like so called promoters[24] that are able to enhance the rate of electrode reactions of proteins such as cytochrome *c* and [4Fe-4S] ferredoxin. It has been speculated for pyridine derivatives as promoters for cytochrome *c*[25] that hydrogen bondings are formed between the promoters and proteins to align the orientation of the proteins on the electrode surface so that rapid electron transfers can occur. Similar events seem to occur if the adsorbed PQQ is bound to FDH.

Adsorption Behavior of PQQ on the Glassy Carbon Electrode. Figure 4a shows a cyclic voltammogram of CO_2-free phosphate buffer containing 5 mmol dm^{-3} PQQ, and parts b and c of Figure 4 show those obtained after electrolysis at –0.7 V *vs* SCE for 10 and 20 h, respectively. It is seen by the electrolysis that the original redox waves of PQQ were gradually changed into quite different ones to give a couple of new redox waves at around –0.6 to –0.7 V *vs* SCE, the results being similar to those obtained at CO_2-saturated phosphate buffer solution containing PQQ and FDH (see Figure 3). If cyclic voltammetry was applied using a freshly prepared electrode with 5 mmol dm^{-3} PQQ solution which was electrolyzed for 20 h at –0.7 V *vs* SCE, the obtained voltammogram was almost the same as those obtained for the

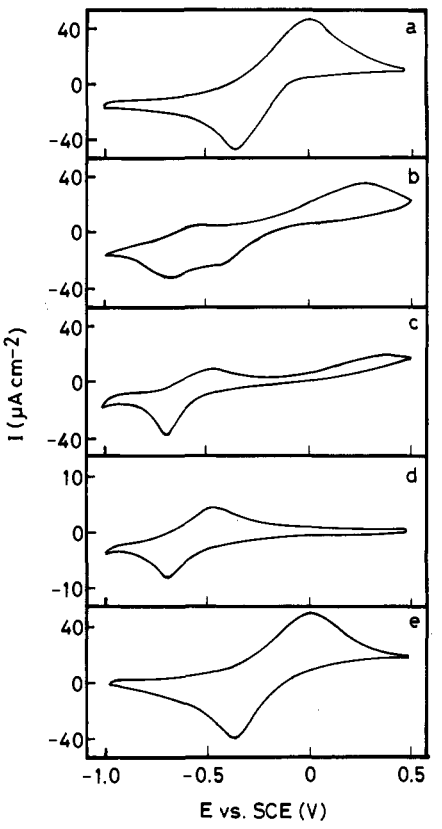

Figure 4. Cyclic voltammograms of a glassy carbon electrode taken in 5 mL of CO_2-free phosphate buffer solution containing 5 mmol dm^{-3} PQQ after electrolysis in the same solution at –0.7 V *vs* SCE for 0 (a), 10 (b), and 20 (c) h. Part d is for the electrode used for 20 h of electrolysis but the voltammogram was taken in freshly prepared phosphate buffer solution containing no PQQ, and part e is for a freshly prepared glassy carbon electrode in the electrolyte solution containing PQQ that was subjected to 20 h of electrolysis. dE/dt = 5 mV s^{-1}.

freshly prepared PQQ solution, as shown in Figure 4e; no reduction wave appeared at around –0.7 *vs* SCE. In contrast, if a cyclic voltammogram was taken in freshly prepared PQQ-free phosphate buffer solution using the glassy carbon electrode that was used for the potentiostatic electrolysis at –0.7 V *vs* SCE for 20 h in the PQQ solution, a reduction wave appeared at around –0.7 V *vs* SCE as shown in Figure 4d, and the voltammogram obtained was quite similar to that given in Figure 4c. It is suggested from these results that PQQ molecules were adsorbed on the glassy carbon electrode during the course of the electrolysis, causing a large negative shift of its redox potential. It is well-known that glassy carbon electrodes have an appreciable amount of functional groups such as hydroxyl, carboxyl, and carbonyl groups on the surface, even if the electrode is exhaustively polished with alumina slurries.[26] These functional groups are often involved in chemisorption of several kinds of organic species[27] and enzymes[28] on glassy carbon electrodes. In the present case, the reduced form of PQQ must have some interaction with the functional groups on the electrode surface.

In order to clarify the chemical nature of the adsorbed PQQ molecules, attempts were made to analyze glassy carbon electrode surfaces used for the electrolysis of the PQQ solution at –0.7 V *vs* SCE for 20 h using a microscopic FT-IR spectrophotometer (Japan Electronic and Optical Ltd., JIR-AQS20M). To prepare

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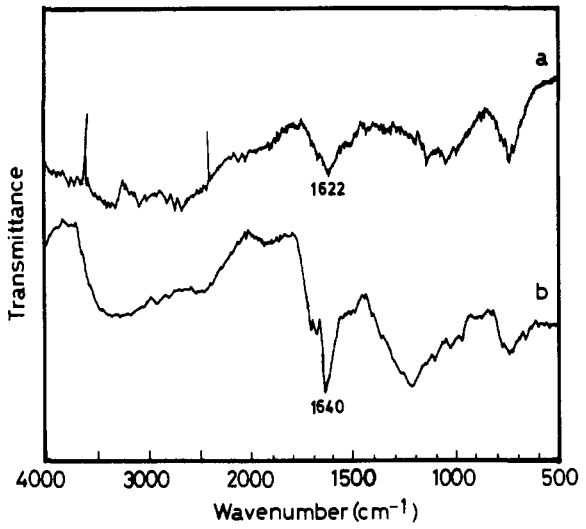

Figure 5. Microscopic IR spectra of the glassy carbon electrode surface obtained after electrolysis at –0.7 V *vs* SCE of phosphate buffer containing 5 mmol dm^{-3} PQQ for 20 h (a) and a PQQ-dispersed KBr disk (b).

the specimen the electrode used for the electrolysis of PQQ was rinsed with distilled water three times to get rid of physically adsorbed PQQ, followed by drying in vacuo for 6 h. The spectrum obtained is shown in part a of Figure 5, together with that obtained for a PQQ-dispersed KBr disk (spectrum b) for comparison. As shown in spectrum b of Figure 5, an intense absorption band which is assigned to quinoid carbonyl stretching appears at 1640 cm^{-1} for PQQ. It is found that the spectrum of the glassy carbon electrode is essentially the same as that of PQQ molecules, indicating that the adsorbed PQQ was present on the electrode surface and its fundamental structure was not largely different from that of free PQQ molecules. It is well-known[29,30] that carbon atoms of quinoid carbonyl groups of PQQ possess high reactivities for nucleophilic reagents, such as acetone, alcohols, and amines, to result in easy formation of their adducts on the carbon atoms with cleavage of the C=O bond. In the case of PQQ adsorbed on glassy carbon, it seems unlikely that these kinds of reactions took place, as judged from the presence of the absorption due to the carbonyl stretching in the FT-IR spectrum. However, it is clearly shown that the IR band of the carbonyl stretching is shifted to a lower wavenumber of 1622 cm^{-1}, suggesting that the C=O distance of the carbonyl groups was elongated.

In general, if delocalized negative charges are generated on a molecule having carbonyl groups, elongation of the C=O distance occurs. One promising source for generation of such negative charges on a PQQ molecule is its deprotonation. Judging from pK_a values of carboxyl groups (pK_a = 1.6, 2.2, 3.3) and the pyrrole moiety (pK_a = 10.3) of PQQ,[22] all carboxyl groups are deprotonated in aqueous solution at pH 7, but the pyrrole moiety is not, suggesting that the observed shift of the IR band results from deprotonation of the pyrrole moiety. Dissociation of hydrogen from nitrogen of the pyrrole moiety of PQQ must occur during the potentiostatic electrolysis at –0.7 V *vs* SCE, resulting in adsorption of PQQ on the glassy carbon electrode through its negatively charged nitrogen. If this is the case, the redox potential of PQQ must be negatively shifted as predicted by Hammett's rule.[31] Such a large negative shift of the redox potential caused by the deprotonation of the pyrrole moiety of PQQ as observed in the present study is rationalized by recent work with the trimethyl ester of PQQ (TMPQQ). The deprotonation of the pyrrole moiety of TMPQQ occurs in acetonitrile with addition of basic reagents such as tetraethylamine[32] and causes changes in the redox potential from –0.07 to –0.87 V *vs* SCE.[33] Considering this finding, the negative shift of the reduction potential of PQQ caused by the adsorption onto the glassy carbon electrode is not unreasonable if the deprotonation of the pyrrole moiety of PQQ occurs as suggested from the FT-IR spectrum.

Table 2. Electrochemical Reduction of Formate[a] Catalyzed by Methanol Dehydrogenase (E.C. 1.1.99.8) with the Use of MV^{2+} or PQQ as an Electron Mediator

electron mediator	concn of mediator (mmol dm^{-3})	amount of MDH (unit)	product and current eff: formaldehyde μmol	formaldehyde %	methanol μmol	methanol %
MV^{2+}	1	12.5	0.32	93	0	0
MV^{2+}	5	0.25	0.08	90	0	0
MV^{2+}	5	2.5	0.35	87	0	0
MV^{2+}	5	12.5	1.9	39	1.1	45
PQQ	0.1	2.5	0	0	0.1	91
PQQ	1	2.5	0	0	1.4	94
PQQ	5	2.5	0	0	1.3	92
PQQ	5	12.5	0	0	0.4	92

[a] The electrolyte solution used was 5 mL of phosphate buffer (pH 7) containing 10 mmol dm^{-3} HCOONa. The electrolysis was performed at –0.7 V *vs* SCE for 20 h using a glassy carbon electrode having 1 cm^2 area.

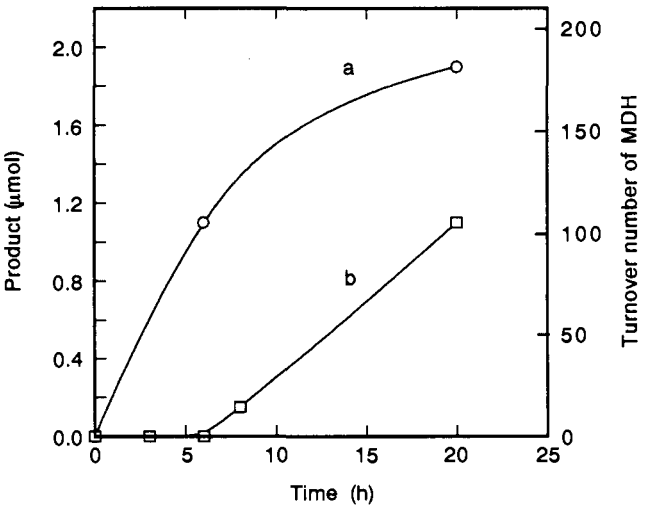

Figure 6. Production of formaldehyde (a) and methanol (b) by electrochemical reduction at –0.7 V *vs* SCE of 5 mL of CO_2-free phosphate buffer solution containing 10 mmol dm^{-3} HCOONa, 12.5 units of MDH, and 5 mmol dm^{-3} MV^{2+}.

Electrochemical Reduction of Formate to Methanol with MDH. Electrolysis at –0.7 V *vs* SCE of CO_2-free phosphate buffer solution containing HCOONa, MDH, and either MV^{2+} or PQQ as the electron mediator for 20 h under nitrogen atmosphere gave the results shown in Table 2. When MV^{2+} was used, formaldehyde was produced as the major reduction product, and its production was enhanced with an increase in the amount of both MV^{2+} and MDH in the electrolyte solution. As long as the results obtained by 20 h of electrolysis are concerned, methanol production occurred only when relatively high concentrations of MDH (12.5 unit) and MV^{2+} (5 mmol dm^{-3}) were used. Figure 6 shows the time course of the formaldehyde and methanol production obtained in that case. The formaldehyde production occurred from the beginning of the electrolysis, whereas the methanol production began to occur when formaldehyde was accumulated to give *ca.* 1.1 μmol whose concentration was 0.22 mmol dm^{-3}. These results as well as the results given in Table 2 indicate that

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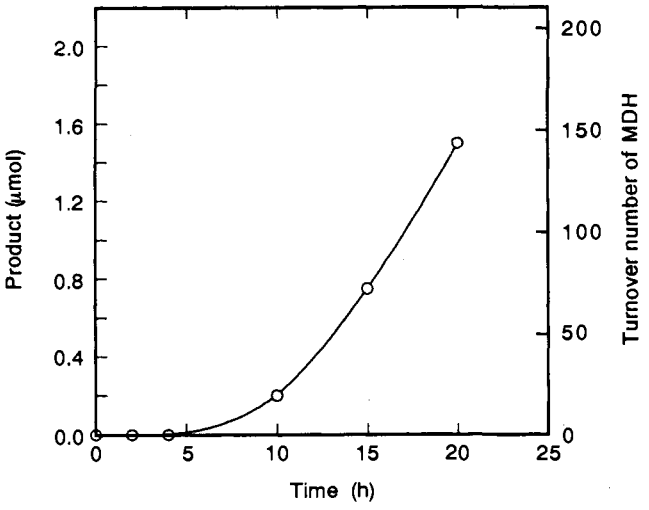

Figure 7. Production of methanol by electrochemical reduction at –0.7 V *vs* SCE of 5 mL of CO_2-free phosphate buffer solution containing 10 mmol dm^{-3} HCOONa, 2.5 units of MDH, and 5 mmol dm^{-3} PQQ.

the accumulation of formaldehyde is essential for the subsequent reduction of formaldehyde to methanol. The following control experiments confirmed the importance of the accumulation of formaldehyde for methanol production. Electrolysis at –0.7 V *vs* SCE of CO_2-free phosphate buffer solution containing 12.5 units of MDH, 5 mmol dm^{-3} MV^{2+}, and 0.1 mmol formaldehyde did not yield methanol at all even if the electrolysis was continued for 30 h, whereas electrolysis under the same conditions except for the concentration of formaldehyde which was increased from 0.1 to 0.5 mmol dm^{-3} yielded methanol without any induction period and its amount increased linearly to 1.5 μmol with electrolysis for 20 h. It is suggested from these results that there is a threshold concentration of formaldehyde between 0.1 and 0.5 mmol beyond which the reduction to methanol easily proceeds.

Being different from MV^{2+}, the use of PQQ exclusively yielded methanol with current efficiencies greater than 90% under all conditions shown in Table 2. No formaldehyde was produced. The time course of the methanol production given in Figure 7 shows the presence of an induction period before the methanol production began to occur. Then it is again suggested that the adsorption of PQQ molecules on the glassy carbon electrode plays a key role in the reduction of formate to methanol, because the thermodynamic potential for the reduction of formate to methanol at pH 7 is –0.56 V *vs* SCE. In fact, the cyclic voltammogram obtained after 20 h of electrolysis was essentially the same as that shown in Figure 4c. The adsorbed PQQ gave irreversible waves, making it difficult to deduce its redox potential, but according to theories of cyclic voltammetry, the redox potential of an irreversible reaction system is more positive than the peak potential of cathodic currents (–0.68 *vs* SCE). Accordingly, arguments based on thermodynamics may predict that the adsorbed PQQ may reduce formate to methanol but not to formaldehyde, because the reduction potential of formate to formaldehyde is –0.68 V *vs* SCE,[23] being comparable to the cathode peak potential.

By comparing the electrolysis results shown in Table 2 for cases with 2.5 units of MDH, increases in the concentration of PQQ from 0.1 to 1 mmol dm^{-3} enhanced the methanol production but further increase to 5 mmol dm^{-3} did not. This result may imply that the amount of PQQ adsorbed on the electrode is saturated for a concentration of PQQ more than 1 mmol dm^{-3}. If the amount of MDH was increased from 2.5 to 12.5 units with the same concentration of PQQ of 5 mmol dm^{-3}, the methanol production was decreased, suggesting that an increase in the enzyme may enhance its adsorption on the electrode, resulting in a decrease of the adsorbed PQQ on the electrode surface.

Reduction of Carbon Dioxide to Methanol with Cooperative Assistance of FDH and MDH. Based on the results shown in

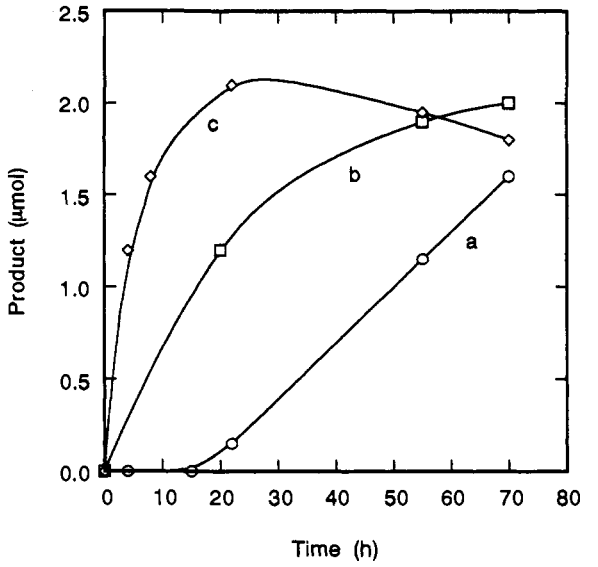

Figure 8. Time course of production of methanol (a), formaldehyde (b), and formate (c) by electrochemical reduction at –0.8 V *vs* SCE of 5 mL of CO_2-saturated phosphate buffer solution containing 2.5 units of FDH, 12.5 units of MDH, 5 mmol dm^{-3} MV^{2+}, and 0.3 mol dm^{-3} $NaHCO_3$.

Figures 1 and 6, the potentiostatic electrolysis at –0.8 V *vs* SCE of CO_2-saturated phosphate buffer solutions containing 0.3 mol dm^{-3} $NaHCO_3$, 2.5 units of FDH, 12.5 units of MDH, and 5 mmol dm^{-3} MV^{2+} was attempted. The results obtained are given in Figure 8. As expected, the formate production took place from the beginning of the electrolysis. Formaldehyde was also produced at a slightly low rate. When the amount of formaldehyde exceeded *ca.* 1 μmol (0.2 mmol dm^{-3}), the methanol production began. The current efficiency determined at 70 h electrolysis was 18% for formate, 37% for formaldehyde, and 40% for methanol. If the concentration of MDH was decreased from 12.5 to 2.5 units without changing the concentration of the other substances, no methanol production was seen, and 4.6 μmol of formate and 0.38 μmol of formaldehyde were produced by 70 h of electrolysis. The results obtained here qualitatively agree with the results given in Figures 1 and 6. Then the consecutive reduction of carbon dioxide to methanol *via* formate and formaldehyde can be postulated for the use of MV^{2+} as the electron mediator.

The use of PQQ as the mediator gave remarkably different results, as shown in Figure 9, which were obtained by electrolysis at –0.8 V *vs* SCE of CO_2-saturated phosphate buffer solution containing 5 mmol dm^{-3} PQQ as the mediator in the presence of 2.5 units of FDH and 2.5 units of MDH. As shown in this figure, methanol alone was produced after some induction period in the electrolysis. The current efficiency obtained for the methanol production was 89% for 70 h of electrolysis. No other reduction product was detected in both gas and liquid phases. The amount of methanol produced was linearly increased by electrolysis for 5 days, but a gradual decrease in the reaction rate was observed with further electrolysis. Decreases in the activities of FDH and/or MDH and predominance in the spontaneous oxidation of methanol at MDH are conceivable as the most likely causes for the observed decrease in the reaction rate. Considering that CO_2 is reduced to formate at FDH with the assistance of PQQ, as shown in Figure 2, the formate production may be expected, but it was not true when MDH was present together with FDH. The observed highly selective methanol production may be indebted to the competitive enzyme reactions of FDH and MDH with the assistance of PQQ molecules adsorbed on the glassy carbon electrode. As mentioned above, both FDH and MDH must be very close to the adsorbed PQQ when the respective enzymatic reactions take place with the assistance of the adsorbed PQQ. An increase in the occupation of the adsorbed PQQ by MDH causes

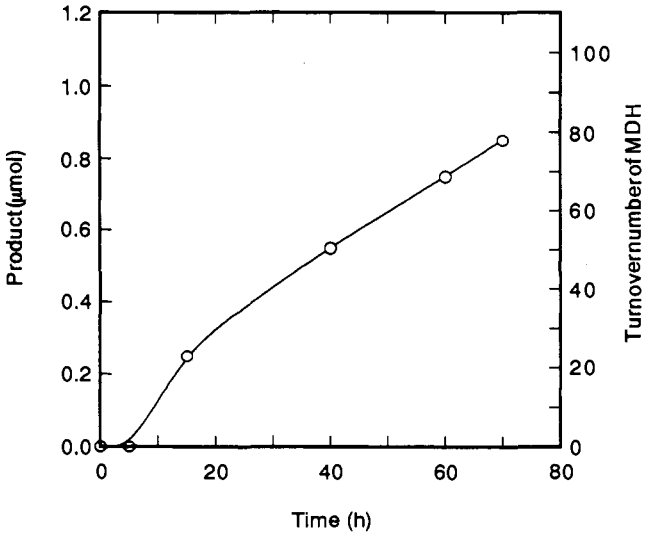

Figure 9. Time course of methanol production by electrochemical reduction at –0.8 V *vs* SCE of 5 mL of CO_2-saturated phosphate buffer solution containing 2.5 units of FDH, 2.5 units of MDH, 5 mmol dm^{-3} PQQ, and 0.3 mol dm^{-3} $NaHCO_3$.

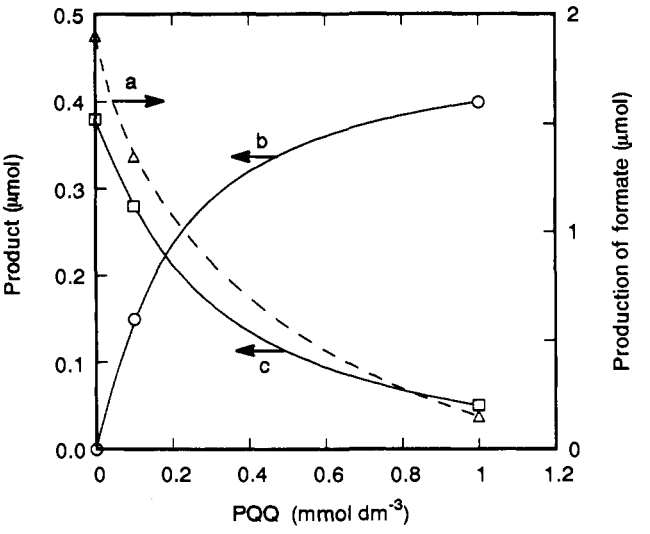

Figure 10. Effects of addition of PQQ on the amount of formate (a), methanol (b), and formaldehyde (c) production obtained by electrolysis at –0.8 V *vs* SCE for 70 h of 5 mL of CO_2-saturated phosphate buffer solutions containing 2.5 units of FDH, 2.5 units of MDH, 5 mmol dm^{-3} MV^{2+}, and 0.3 mol dm^{-3} $NaHCO_3$.

a decrease in that by FDH, resulting in a decrease in the reduction rate of CO_2 to formate. When formate is produced at FDH it may easily be trapped by the neighboring MDH molecules where the subsequent reduction of formate to methanol takes place, resulting in the selective reduction of carbon dioxide to methanol, as shown in Figure 9.

The role of the adsorbed PQQ molecules in the electrochemical reduction of carbon dioxide to methanol in this system was indirectly confirmed by electrolysis for 70 h at –0.8 V *vs* SCE of carbon dioxide in the presence of both MV^{2+} and PQQ as electron mediators together with two kinds of enzymes. The amount of FDH and MDH was 2.5 units each in 5 mL of the CO_2-saturated phosphate buffer solution. The concentration of MV^{2+} was fixed to 5 mmol dm^{-3}, and 0, 0.1, or 1.0 mmol dm^{-3} PQQ was added to the electrolyte solution. As shown in Figure 10, the use of MV^{2+} alone gave formate and formaldehyde as the reduction products of CO_2, as already described above. The production of methanol became significant with an increase in the addition of PQQ, but the production of both formate and formaldehyde was suppressed, and nearly complete suppression was achieved when PQQ was added so as to give one-fifth the concentration of MV^{2+}. The adsorption of PQQ on the electrode surface must hinder the reduction of MV^{2+}, resulting in a decrease in abilities of MV^{2+} for mediation for FDH and MDH. In fact, it was observed that the blue coloring of the electrolyte solution due to production of $MV^{\bullet+}$ became weak with an increase in the concentration of added PQQ.

As described above, the type of mediator greatly influenced the reduction behavior of carbon dioxide. In general, the rate of enzyme reaction is greatly influenced by the kind of mediator used, as demonstrated, for example, in amperometric detection of glucose. Among a variety of redox agents tested as mediators for glucose oxidase, $[Os(bpy)_2Cl]^{2+}$-attached poly(vinylpyridine)[34] has more than several hundred times greater ability for the electron mediation than common redox mediators such as ferrocene and quinone compounds. Accordingly, an improvement in the rate of electrochemical synthesis of methanol from carbon dioxide may be achieved if appropriate mediators are discovered.

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