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# Electrochemical Fixation of $CO_2$ in Acetyl-coenzyme A to Yield Pyruvic Acid Using Pyruvate Dehydrogenase Complexes as an Electrocatalyst

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Electrochemical fixation of $CO_2$ in acetyl-coenzyme A has successfully been accomplished by using pyruvate dehydrogenase complex as an electrocatalyst. The production of pyruvic acid was enhanced when coenzyme A, which was a byproduct in the pyruvic acid production, was converted to the substrate in situ in the electrolytic cell with assistance of acetyl phosphate and phosphotransacetylase.

Conversion of $CO_2$ into useful chemicals have become very popular in recent years. So far direct electrolytic reduction of it to CO, HCOOH, HCHO, $(COOH)_2$, $CH_4$ etc. has most extensively been investigated by using a variety of electrodes and electrocatalysts. In contrast, few studies have been done on electrochemical fixation of $CO_2$ in organic molecules; electrolytic reduction of 1,4-benzoquinone in the presence of $CO_2$ resulted in 2,5-dihydrobenzoic acid[1] and that of ethylthioacetate in the presence of molybdenum-iron cluster and $CO_2$ yielded pyruvic acid.[2] Recently, we have reported the electrochemical fixation of $CO_2$ in oxoglutaric acid to yield isocitric acid by using isocitrate dehydrogenase as an electrocatalyst.[3]

The pyruvate dehydrogenase complex (PDC) is an enzyme which catalyzes oxidative decarboxylation of pyruvic acid to acetyl-coenzyme A (acetyl-SCoA) in vivo[4] as given by

$$CH_3\text{-}\underset{\underset{O}{\|}}{C}\text{-}COO^- + HSCoA + NADP^+ \xrightarrow[PDH]{} CH_3\text{-}\underset{\underset{O}{\|}}{C}\text{-}SCoA + CO_2 + NADPH \qquad (1)$$

where HSCoA and $NADP^+$ are coenzyme A and nicotinamide-adenine dinucleotide phosphate, respectively. It is well known[5,6] that the reverse reaction giving reductive carboxylation of acetyl-SCoA occurs chemically or photochemically if one uses enzymes such as PDC and pyruvate-ferredoxin oxidoreductase. In the present study, the reverse reaction was electrochemically

undertaken by using PDC as an electrocatalyst and methyl viologen ($MV^{2+}$) as an electron mediator. The reaction scheme postulated here is given by dotted square in Fig. 1. The electrochemical reduction was carried out potentiostatically by using an H-type cell separated by a Nafion membrane. A glassy-carbon (GC) plate and a Pt plate were used as a cathode and an anode, respectively, and a saturated calomel electrode (SCE) served as a reference electrode. The cathode compartment contained 10 ml of $CO_2$-saturated 0.15 mol $dm^{-3}$ phosphate buffer solution (pH 5) containing $1.5 \times 10^{-2}$ mol $dm^{-3}$ acetyl-SCoA, 1 unit of PDC, $6.6 \times 10^{-3}$ mol $dm^{-3}$ $MV^{2+}$, $3.3 \times 10^{-3}$ mol $dm^{-3}$ $NaHCO_3$, and $2.5 \times 10^{-2}$ mol $dm^{-3}$ 2-mercaptoethanol which served as a stabilizer of the enzyme, while the anode compartment contained the phosphate buffer alone. Production analysis was carried out using a high pressure liquid chromatograph equipped with an UV detector and an organic acid column (Waters). Figure 2 shows the time course of the pyruvic acid production at -0.95 V vs. SCE which is negative enough to reduce $MV^{2+}$ to $MV^{+}$. The result shows that $MV^{+}$ generated at GC electrode carried electrons effectively to PDC where the fixation of $CO_2$ in acetyl-SCoA took place. Pyruvic acid was increased up to 0.2 μmol for the first 10 h of the electrolysis, followed by stagnation in its production. In the production of pyruvic acid, HSCoA must be produced in the equivalent amount, as suggested by Eq. 1 , but this substance inhibits the occurrence of the reverse reaction.[5] The saturation tendency for the pyruvic acid production is then judged to be reasonable from the viewpoint of the accumulation of HSCoA. Indeed, it was observed that no pyruvic acid was produced by electrolysis for 100 h of the above mentioned electrolyte solution if 0.5 μmol of acetyl-SCoA was added prior to the electrolysis. The molecular weight of PDC is ca. $9 \times 10^6$, and one PDC contains 30 active centers on average.[4] In the present experiments, 1 unit of PDC was dissolved in the electrolyte solution in which 0.6 mg of PDC molecules was contained. Accordingly, the amount of active centers dissolved in the solution is judged to be $2 \times 10^{-9}$ mol which was ca. one hun-

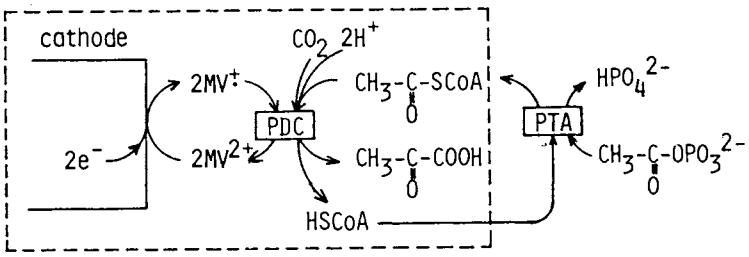


Fig. 1. Reaction scheme postulated in this study.

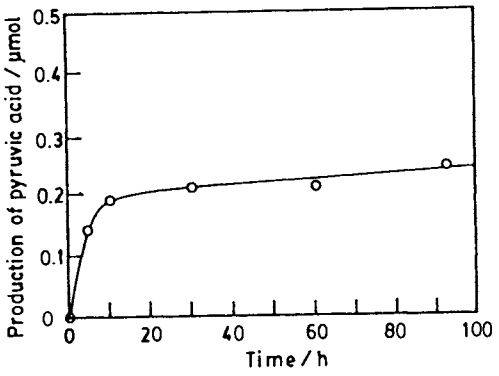


Fig. 2. Production of pyruvic acid by electrochemical fixation in acetyl-SCoA as a reaction substrate.

dredth of the amount of pyruvic acid produced. Then, it is concluded that the reverse reaction was strongly inhibited when the amount of HSCoA was increased to ca. 100 times as much as that of active centers of PDC.

Accordingly, it is required to eliminate the produced HSCoA from the electrolytic cell to enhance the electrolytic production of pyruvic acid. We have accomplished this by using enzymatic reaction of HSCoA with acetyl phosphate in the presence of phosphotransacetylase (PTA) to result in acetyl-SCoA. It was found that the rate of this enzymatic reaction was fairly high. If 5 units of PTA were added to 5 ml of $CO_2$-saturated 0.15 mol $dm^{-3}$ phosphate buffer solution (pH 5) containing $1.0 \times 10^{-4}$ mol $dm^{-3}$ HSCoA, $1.0 \times 10^{-4}$ mol $dm^{-3}$ acetyl phosphate, and $3.3 \times 10^{-3}$ mol $dm^{-3}$ $NaHCO_3$, the production of acetyl-SCoA was completed for ca. 30 min as judged from changes in absorbance at 230 nm which is characteristics of the thioether group that is contained in acetyl-SCoA produced.

By adding PTA and acetyl phosphate to the electrolytic system used in the $CO_2$ fixation experiments whose results are shown in Fig. 2, we can construct the overall reaction schemes as shown in Fig. 1. The electrolysis was carried out at -0.95 V vs. SCE using the electrolyte solution which contained $1.7 \times 10^{-4}$ mol $dm^{-3}$ HSCoA, $1.5 \times 10^{-2}$ mol $dm^{-3}$ acetyl phosphate, and 25 units of PTA instead of acetyl-SCoA. The result is shown in Fig. 3. By comparing the results shown in this figure with that given by Fig. 2, it is noticed that the production of pyruvic acid was enhanced by almost five times as a result of converting HSCoA to acetyl-SCoA. In Fig. 3 the turnover number is given which was estimated based on the ratio of the amount of pyruvic acid produced to that of active centers of PDC ($2.0 \times 10^{-9}$ mol). The turnover number amounted to ca. 500, indicating that the electrochemical fixation of $CO_2$ in acetyl-SCoA occurred without serious inhibition of HSCoA. However, even in that case, a saturation tendency of pyruvic acid production was observed after 50 h.

The electrochemical fixation of $CO_2$ was attempted under several different pHs and electrode potentials using the same electrolyte solution as that mentioned above. It was observed that in all cases, the production of pyruvic acid proceeded linearly with the electrolysis time in the initial stage of the electrolysis, followed by saturation, the behavior being similar to

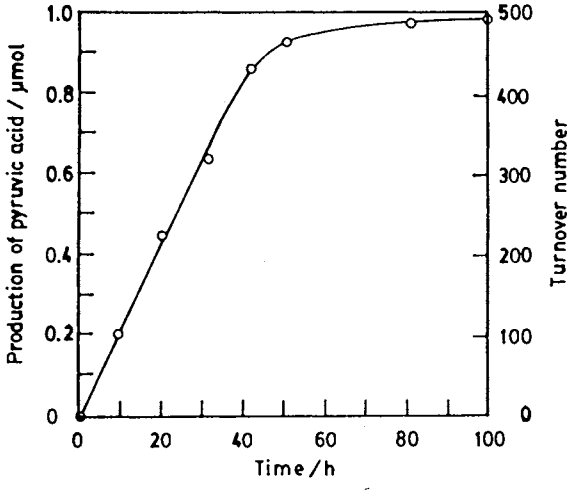


Fig. 3. Production of pyruvic acid by electrochemical fixation in acetyl-SCoA that result from acetyl phosphate with HSCoA.

that shown in Fig. 3. Table 1 summarizes reaction rate obtained for the initial 10 h of electrolysis and the amount of pyruvic acid produced by the electrolysis for 100 h where the production of pyruvic acid was eventually stagnated. The highest reaction rate and the largest amount of production of pyruvic acid were obtained for electrolysis at pH = 5.0 and E = -0.95 V vs. SCE. It is suggested from the effect of the electrode potential on the rate of reaction that methyl viologen anion radicals mediate the pyruvic acid production as illustrated in Fig. 1. Another interesting finding was that if 1 μmol of pyruvic acid was added in the electrolyte solution prior to electrolysis, the electrolysis did not result in any remarkable production of pyruvic acid. This result seems to suggest that pyruvic acid has an unfavorable effect of inhibiting the $CO_2$ fixation like HSCoA, if its amount was much larger than that of the active centers of PDC. The saturation in the production of pyruvic acid observed in Fig. 3 may be due to at least in part to the accumulation of pyruvic acid.

Table 1. Electrochemical fixation of $CO_2$ into acetyl-SCoA to yield pyruvic acid

| E / V vs. SCE | pH | Reaction rate / $10^{-9}$ mol $h^{-1}$ | Amount produced at 100 h / μmol |
|---|---|---|---|
| -0.95 | 3.0 | 11 | 0.56 |
| -0.95 | 5.0 | 20 | 0.98 |
| -0.95 | 7.0 | 12 | 0.55 |
| -0.75 | 5.0 | 17 | 0.79 |
| -0.85 | 5.0 | 19 | 0.86 |
| -1.05 | 5.0 | 16 | 0.65 |
| -0.95[a)] | 5.0 | 0 | 1.0 |

a) 1.0 μmol of pyruvic acid was previously added in the electrolyte solution.

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