Kinetic studies of the interaction between diphenylviologen derivative as a novel co-enzyme and malic enzyme for building carbon-carbon bond from CO_2

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Abstract: Malic enzyme (ME) catalyzes a reaction of introducing CO_2 as a carboxy group to pyruvate (C3) to form malate (C4) via oxaloacetate in the presence of co-enzyme NADPH. Recently, we reported that multi-electron reduced form of diphenylviologen (PV) act as an electron mediator for ME. However, an interaction between PV and ME has not been clarified. In this study, we investigated the reaction of introducing CO_2 to pyruvate with PV derivative and ME. The concentration of oxaloacetate production was determined and the affinity between PV derivative and ME was estimated by kinetic analysis with Michaelis-Menten equation.

Keywords: Carbon-carbon bond formation, Malic enzyme, Artificial co-enzyme

1. Introduction

Recently, there are some global environment and resource problems. To solve these problems, solar energy-based CO_2 utilization technologies including artificial photosynthesis have been received a lot of attention. As one of these technologies, visible-light-driven CO_2 reduction to CO or formate system consisted of a photosensitizer, an electron mediator and a biocatalyst was developed.[1] To use CO_2 as a feedstock, effective catalyst for carbon-carbon bond formation from CO_2 is necessary. In various types of catalysts, a biocatalyst is a useful catalyst because it has high selectivity for product in aqueous solution at room temperature and ordinary pressure. For example, malic enzyme (ME) catalyzes a reaction of introducing CO_2 as a carboxy group to pyruvate (C3 compound) to form malate (C4 compound) via oxaloacetate in the presence of natural co-enzyme NADPH. We reported visible-light-driven malate production system consisted of ME and diphenylviologen (PV, Figure 2) instead of NADPH (Figure 1). In the system, multi-electron reduced PV was produced with photosensitization of water-soluble porphyrin (TPPS) and it act as an electron mediator for ME.[2,3] However, an interaction between ME and multi-electron reduced PV has not been clarified.

In this study, PV derivative, 1,1'-bis(*p*-carboxyphenyl)-4,4'-bipyridiniu dichloride (PCV, Figure 2) was synthesized and electrochemical properties of PCV were investigated. In addition, we researched the reaction of introducing CO_2 to pyruvate with the multi-electron reduced PCV and ME by enzyme kinetic analysis.

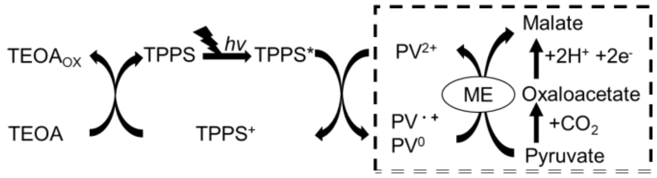

Figure 1. Visible-light-driven malate production system consisting of an electron donor (TEOA), a photosensitizer (TPPS), PV and ME.

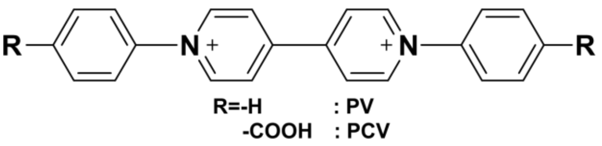

Figure 2. Chemical structures of diphenylviologen derivatives (PV and PCV).

2. Experimental

PCV was synthesizes according to previous reported method with some modication. For the electrochemical study, the reduction potentials of PCV were measured by cyclic voltammetry with a carbon electrode as a working, a platinum electrode as a counter and a Ag/AgCl electrode as a reference in 0.2 M KCl solution.

The enzyme kinetic analysis on the reaction of introducing CO_2 to pyruvate to form oxaloacetate was carried out according to the following method. The sample solution consisted of pyruvate (1.2 mM), bicarbonate (1.2 mM), Mg^{2+} (1.0 mM) as a co-factor for ME and PCV. In this reaction, sodium dithionite (1.1 mM) was used as a reducer for PCV. The solution was de-aerated and flushed argon gas. Then, reaction was started by injecting ME (4 units). The concentration of oxaloacetate production after 60 min incubation was determined by ion chromatograph and plotted against the concentration of double-electron reduced PCV (PCV^0). The kinetic parameters for oxaloacetate production with PCV^0 and ME were determined with Michaelis-Menten equation.

3. Results and discussion

Electrochemical properties of PCV was investigated by measuring of reduction potential of PCV with cyclic voltammetry. The single- and double-electron reduction potentials of PCV are estimated to be -0.42 and -0.70 V (vs. Ag/AgCl), respectively. There is little difference in reduction potentials compared with PV (-0.39 and -0.74 V, vs. Ag/AgCl). This indicates that the carboxy group bonded to 4,4'-bipyridinium site via phenyl group is not affected to the reduction potential.

Figure 3 shows the relationship between the concentration of PCV^0 and the rate of oxaloacetate production. The initial rate of oxaloacetate production increased with increasing the concentration of PCV^0 and then reached constant value. The reaction of introducing CO_2 to pyruvate with the PCV^0 and ME was coincided with Michaelis-Menten model. This shows that PCV^0 acts as an artificial co-enzyme for ME. The kinetic parameters for the reaction were calculated by Lineweaver-Burk plot as shown in Table 1.

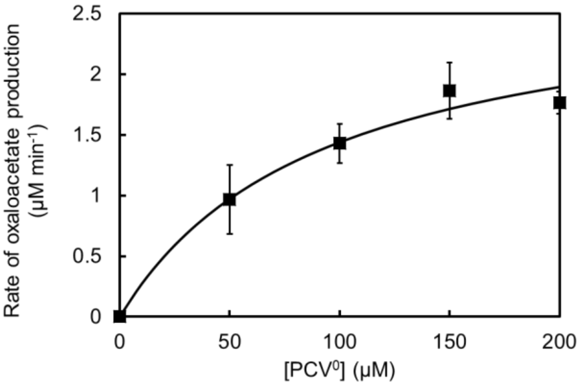

Figure 1. The relationship between the concentration of PCV^0 and the rate of oxaloacetate production.

Table 1. Kinetic parameters for the reaction of introducing CO_2 to pyruvate with PCV^0 and ME.

	V_{max} (μM min^{-1})	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (M^{-1} min^{-1})
PCV^0	2.8	93	3.0	3.2×10^4

The kinetic parameters V_{max}, K_m and k_{cat} were determined by fitting to Michaelis-Menten equation. The molecular weight of ME from chicken liver was estimated to be 5.6×10^4.[4]

4. Conclusions

PCV was synthesized and electrochemical properties of PCV was studied. Reduction potentials of PCV were little difference compared with those of PV. In addition, the interaction between PCV and ME was investigated. We found that PCV^0 act as a co-enzyme for ME. The kinetic parameters for oxaloacetate production from CO_2 and pyruvate with PCV^0 and ME were determined for the first time. These findings help to improve the catalytic activity of ME for carbon-carbon bond formation from CO_2 as a feedstock by developing a novel viologen derivative.

References

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