Selective formate reduction to formaldehyde with aldehyde dehydrogenase and single-electron reduced methylviologen

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Abstract: CO_2 is converted to methanol via formate and formaldehyde with single-electron reduced methylviologen ($MV^{\cdot+}$) as an artificial co-enzyme, formate (FDH), aldehyde (AldDH) and alcohol dehydrogenases (ADH). The enzyme kinetic parameters for FDH catalyzing CO_2 conversion to formate with $MV^{\cdot+}$ or ADH catalyzing formaldehyde conversion to methanol with $MV^{\cdot+}$ have been clarified. However, the kinetic parameters for AldDH catalyzing formate conversion to formaldehyde with $MV^{\cdot+}$ have not been clarified. In this study, the parameters for formate reduction with $MV^{\cdot+}$ and AldDH were determined by enzyme kinetic analysis.

Keywords: Kinetic analysis, Artificial co-enzyme, Aldehyde dehydrogenase.

1. Introduction

Currently, global warming due to an increase CO_2 and exhaustion of fossil fuels are problems. As a method to solve these problems, CO_2 reduction system with visible light has been proposed. CO_2 is reduced to methanol via formate and formaldehyde with formate (FDH), aldehyde (AldDH) and alcohol dehydrogenases (ADH) in the presence of single-election reduced methylviologen ($MV^{\cdot+}$) [1]. The enzyme kinetic parameters for CO_2 conversion to formate with $MV^{\cdot+}$ and FDH or formaldehyde reduction to methanol with $MV^{\cdot+}$ and ADH have been clarified [1,2]. However, the kinetic parameters for formate reduction to formaldehyde conversion with $MV^{\cdot+}$ and AldDH have not been revealed. In this study, enzyme kinetic analysis was carried out to elucidate the parameters for formate reduction with $MV^{\cdot+}$ and AldDH to compare with those of FDH, ADH. In addition, whether oxidized methylviologen (MV^{2+}) acts as an artificial co-enzyme for AldDH in formaldehyde oxidation to formate conversion was investigated.

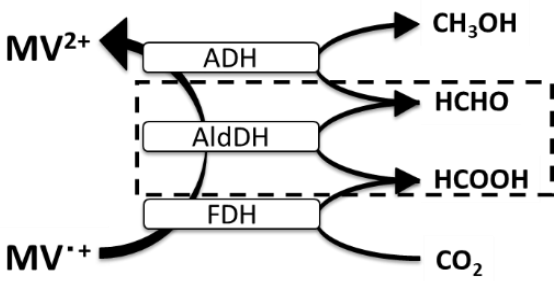

Figure 1. CO_2 - methanol conversion with three dehydrogenases (ADH, AldDH, FDH) and reduced methylviologen

2. Experimental

In the formaldehyde oxidation with AldDH and MV^{2+}, MV^{2+} (100~500 μM), formaldehyde (2.0 mM) and AldDH (1.3 μM) in 1.0 mM sodium pyrophosphate buffer (pH = 7.4) was reacted for 1 min at 30.5 °C in argon atmosphere. The progress of the formaldehyde oxidation was measured from the absorbance of $MV^{\cdot+}$ generated and the molar absorption coefficient (ε_{605} = 12000 M^{-1} cm^{-1}). In the formate reduction to aldehyde with AldDH and $MV^{\cdot+}$, $MV^{\cdot+}$ (25~500 μM), sodium formate (2.0 mM) and AldDH (1.3 μM) in 1.0 mM sodium pyrophosphate buffer (pH = 7.4) was reacted for 1 min at 30.5 °C in argon atmosphere. Sodium dithionite was used as a reducing agent for MV^{2+}. The amount of formate consumption was measured by ion chromatography. The amount of formaldehyde production was estimated as the amount of formate consumption.

3. Results & Discussion

Figure 2 shows the relationship between initial rate for formate production and the concentration of MV^{2+} or NAD^+ as a reference in formaldehyde oxidation with AldDH. $MV^{\cdot+}$ was not produced by progressing of formaldehyde oxidation, indicating that MV^{2+} did not act as a co-enzyme for AldDH. Figure 3 shows the relationship between initial rate for formaldehyde production and the concentration of $MV^{\cdot+}$ or NADH. Since the initial rate formaldehyde production was increased with increasing the concentration of $MV^{\cdot+}$ and then reached a constant value, showing this enzyme reaction was coincide with Michaelis - Menten equation. $MV^{\cdot+}$ acted as an artificial co-enzyme for AldDH in the formate reduction.

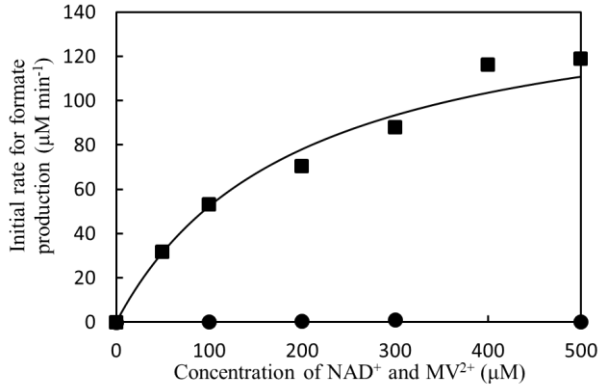

Figure 2. The relationship between the concentrations of MV^{2+} (●), NAD^+ (■) and initial rate for formate

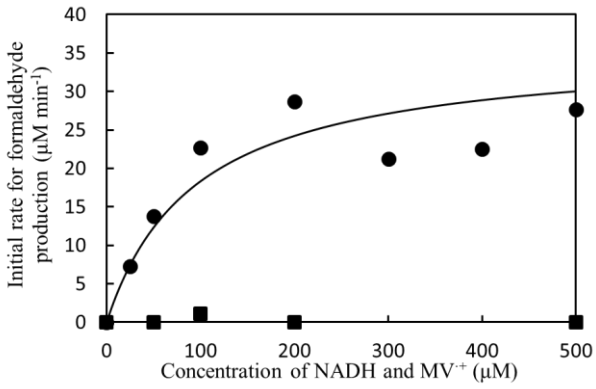

Figure 3. The relationship between the concentrations of $MV^{\cdot+}$ (●), NADH (■) and initial rate for formaldehyde

4. Conclusions

The kinetic parameters for formate reduction to formaldehyde with $MV^{\cdot+}$ and AldDH calculated by Lineweaver - Burk equation. Table 1 shows the kinetic parameters for FDH, AldDH and ADH with $MV^{\cdot+}$. Comparing with the Michaelis constant K_m which is the ease of binding with FDH, AldDH and ADH, the K_m of AldDH is the smallest in other dehydrogenases. It indicated the affinity between $MV^{\cdot+}$ and AldDH is stronger than those of FDH and ADH. Furthermore, the formate reduction catalytic efficiency value k_{cat}/K_m of AldDH was the highest in other dehydrogenases. Based on these parameters, we will construct CO_2 - methanol conversion more efficiency by changing the enzyme concentration ratio of FDH, AldDH and ADH from 1:1:1 to 2:1:3.

Table 1. The enzyme kinetic parameters for dehydrogenases with reduced methylviologen [2,3]

	V_{max}(μM・min^{-1})	K_m(μM)	k_{cat}(min^{-1})	k_{cat}/K_m(M^{-1}・min^{-1})
FDH	17.8	212	1.90	9,000
AldDH	34.1	87.5	25.7	293,000
ADH	7.13	311	3.57	11,400

V_{max} : Maximum velocity, K_m : Michaelis constant, k_{cat} : Turnover frequency, k_{cat}/K_m : Catalytic efficiency

References

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