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Photochemical synthesis of formic acid from CO_2 with formate dehydrogenase and water-soluble zinc porphyrin

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Abstract

Photochemical synthesis of formic acid was investigated from hydrogen carbonate ion with formate dehydrogenase (FDH) from *Saccharomyces cerevisiae* and methylviologen (MV^{2+}) photoreduction by the visible light photosensitisation of zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP) in the presence of triethanolamine (TEOA) as an electron donating reagent. Irradiation of a solution containing TEOA, ZnTMPyP, MV^{2+}, sodium hydrogen carbonate and FDH with visible light resulted in formic acid synthesis. The concentration of formic acid was 62 μM after 3 h irradiation under the condition of 30 μM MV^{2+}, 20 units FDH and pH 8.

Keywords: Photochemical formic acid synthesis; Zinc porphyrin; Formate dehydrogenase; Enzymatic synthesis

1. Introduction

Carbon dioxide fixation is a potential technology for the realization of catalytic CO_2 reduction [1,2]. Many studies on electrocatalysed CO_2 reduction have been performed using specific electrode materials [3]. On the other hand, studies on CO_2 fixation also have investigated photocatalysis on semiconductors such as titanium dioxide, silicone carbide and strontium titanate [4,5]. However, these systems use ultraviolet irradiation and the total reaction is low yield, whereas highly efficient CO_2 fixation system using visible light is more desirable. Visible light-induced photoredox systems consisting of an electron donor, a photosensitiser, and an electron relay, have been widely studied [6]. The effective photosensitiser is an essential component of such systems. Ruthenium(II) coordination compounds and porphyrins are widely used as effective photosensitisers in photoredox systems. As water-soluble zinc porphyrins, zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP), exhibit strong absorption bands in the visible light region (380–600 nm), these porphyrins are more widely used as an effective photosensitiser than ruthenium(II) coordination compounds [7–10]. On the other hand, biological and enzymatic CO_2 fixation systems have also received much attention. For example, CO_2 can be reduced to formic acid with formate dehydrogenase (FDH) and NADH [11]. Therefore, homogeneous photochemical CO_2 reduction system that combines the photoreduction of NAD^+ by the photosensitisation of water-soluble zinc porphyrin and ferredoxin-$NADP^+$ reductase (FDR), and CO_2 reduction with FDH as shown in Scheme 1 can be established. Some studies relative to photochemical CO_2 reduction with enzyme have been reported. The CO_2 fixation system based on malic acid synthesis combined with the photoreduction of NAD^+ by the photosensitisation of chemical-modified chlorophyll and FDR, and malic acid synthesis from pyruvate and CO_2 (hydrogen carbonate ion) with malic enzyme has also been reported [12].

Our investigation first attempted the development of the visible light-induced formic acid synthesis from CO_2 as shown in Scheme 1. However, the conversion yield of NAD^+ to NADH with FDR was very low. Moreover, FDR is a very expensive reagent. If methylviologen (MV^{2+}), the cheaper electron mediator, can be used instead of NAD^+, an effective CO_2 reduction system could be established without requiring FDR and NAD^+ as shown in Scheme 2. Enzymatic formic acid synthesis from CO_2 (hydrogen carbonate ion) with FDH and MV^{2+} photoreduction with a system containing ruthenium(II) coordination compound as a photosensitiser and

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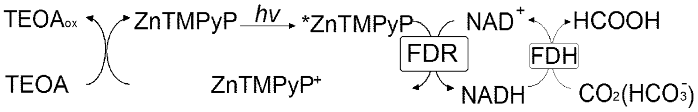
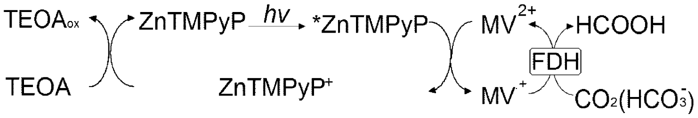


Scheme 1. Photochemical formic acid synthesis from CO_2 (HCO_3^-) with FDH and the photoreduction of NAD^+ with TEOA, ZnTMPyP and FDR.



Scheme 2. Photochemical formic acid synthesis from CO_2 (HCO_3^-) with FDH and the photoreduction of MV^{2+} with TEOA and ZnTMPyP.

mercaptoethanol (RSH) as an electron donor has been reported [13]. In contrast, we previously reported the photochemical formic acid synthesis with FDH and water-soluble zinc porphyrin [14]. However, the detailed reaction of the photochemical formic acid synthesis from CO_2 (hydrogen carbonate ion) with FDH and MV^{2+} photoreduction by water-soluble zinc porphyrin has not been clarified yet.

Here we describe a system for the photochemical formic acid synthesis from CO_2 (hydrogen carbonate ion) with FDH from *Saccharomyces cerevisiae* and reduced MV^{2+} produced by the visible light photosensitisation of ZnTMPyP, in the presence of TEOA as an electron donor and the optimum condition of formic acid synthesis.

2. Experimental

2.1. Materials

FDH from *Saccharomyces cerevisiae* was obtained from Sigma Co. Ltd. NAD^+ was purchased from Oriental Yeast Co. Ltd. Methylviologen dichloride, tetrakis(4-methylpyridyl) porphyrin and triethanolamine were supplied by Tokyo Kasei Co. Ltd. The other chemicals were analytical grade or the highest grade available. One unit of FDH activity was defined as the amount of enzyme required to produce 1.0 μmol formic acid to CO_2 per minute in the presence of NAD^+.

2.2. Preparation of zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP)

Zinc tetrakis(4-methylpyridyl) porphyrin was synthesized by heating TMPyP with about 10 times the molar equivalent of zinc acetate in 100 ml methanol under reflux at 60 °C for 2 h [15]. The synthesis of ZnTMPyP was monitored by UV-Vis absorption spectroscopy using a Shimadzu Multispec 1500 spectrophotometer. During the reaction, the characteristic absorption band of ZnTMPyP at 550 nm increased and the absorbance at 650 nm of TMPyP decreased gradually. The solvent was removed using a vacuum pump and the residue dissolved in water. A solution of ammonium hexafluorophosphate was added to replace the counter-anion with hexafluorophosphate anion. The precipitate of ZnTMPyP hexafluorophosphate salt was collected by suction filtration, washed with water and dried under a vacuum overnight. The ZnTMPyP hexafluorophosphate anion salt dissolved in acetonitrile and tetraethylammonium chloride was added to replace the counter-anion with chloride anion. The precipitate of ZnTMPyP chloride salt was collected by suction filtration, washed with acetonitrile and dried under a vacuum overnight. The product was purified by gel permeation chromatography (Sephadex LH-20; water as elutant).

2.3. Photoreduction of MV^{2+} by photosensitisation of ZnTMPyP

A solution containing ZnTMPyP (9.0 μM), MV^{2+} and TEOA (0.3 M) as an electron donating reagent in 3.0 ml of 10 mM potassium phosphate buffer was deaerated by freeze-pump-thaw cycles repeated six times. The sample solution was irradiated with a 200 W tungsten lamp (Philips) at a distance of 3.0 cm with a Toshiba L-39 cut-off filter at 30 °C. Reduced MV^{2+} ($MV^{\bullet+}$) concentration was determined by the absorbance at 605 nm using the molar coefficient 13,000 $M^{-1}\,cm^{-1}$ [16]. MV^{2+} concentrations in the solution were varied between 15 and 120 μM. The pH value of reaction mixture was adjusted to 7 or 8.

2.4. Visible light-induced formic acid synthesis with FDH and ZnTMPyP

A solution containing ZnTMPyP (9.0 μM), MV^{2+}, TEOA (0.3 M) and FDH was deaerated by freeze-pump-thaw cycles repeated six times and then flushed with argon gas for 5 min. Sodium hydrogen carbonate solution (1 mM) was added to the sample solution and then flushed with argon gas for 5 min. The sample solution was irradiated with a 200 W tungsten lamp and wavelengths of less than 390 nm were blocked with a cut-off filter at 30 °C. The amounts of formic acid and hydrogen carbonate ion were detected by HPLC with an electrical conductivity detector (Shimadzu CDD-10A_{VP}) (column temperature, 40 °C; column, polystyrene sulfonate column Shimadzu SCR-H; elutant, *p*-toluene sulfonic acid; and flow rate, 0.8 ml min^{-1}). FDH activities in a solution were varied between 2.5 and 30 units. MV^{2+} concentrations in the solution were varied between 15 and 120 μM. The pH value of reaction mixture was adjusted to 7 or 8.

3. Results and discussion

3.1. Photoreduction of MV^{2+} with visible light sensitisation of ZnTMPyP

When the reaction mixture containing ZnTMPyP, MV^{2+} and TEOA was irradiated with visible light, the absorbance at 605 nm attributed to the absorption band of $MV^{\bullet+}$ increased with irradiation time. The initial rate (v_0) was determined by the $MV^{\bullet+}$ concentration after irradiation for 10 min. Fig. 1 shows the relationship between the initial rate and MV^{2+} concentrations. The symbols of squares and circles in Fig. 1 are pH 7 and 8 conditions, respectively. The initial rate of $MV^{\bullet+}$ formation increased with the concentration of MV^{2+}. At 120 μM MV^{2+} concentration, the initial rates (v_0) under pH 7 and 8 were 1.3 and 2.0 μM min^{-1}, respectively. Fig. 2 shows the relationship between the yield of MV^{2+} to $MV^{\bullet+}$ ($[MV^{\bullet+}]/[MV^{2+}]$) after 80 min irradiation and MV^{2+} concentrations. The symbols in Fig. 2 are the same as in Fig. 1. In contrast to the relationship between the initial rate and MV^{2+} concentrations, the yield of MV^{2+} to $MV^{\bullet+}$ increased with the concentration of MV^{2+} up to 30 μM and then gradually decreased. The yields under pH 7 and 8 were 40 and 52% at 30 μM MV^{2+}, respectively. From the result of Fig. 1, the turnover number of ZnTMPyP in the MV^{2+} photoreduction is a nearly fixed value of 0.2 μM min^{-1} under the MV^{2+} concentration over 60 μM. Since the reaction efficiency decreases under the MV^{2+} concentration over 60 μM, the yield of MV^{2+} to $MV^{\bullet+}$ also decreases. From the results of Figs. 1 and 2, the MV^{2+} photoreduction was promoted under high pH condition. FDH catalyses both the reduction of hydrogen carbonate ion to formic acid and the oxidation of formic acid to hydrogen carbonate ion. The reaction rate of the reduction of hydrogen carbonate ion to formic acid is 20 times slower than that of the oxidation of formic acid to hydrogen carbonate ion in the system with FDH. At high MV^{2+} concentrations, hydrogen carbonate ion was reproduced by the oxidation of formic acid with FDH. The yield of MV^{2+} to $MV^{\bullet+}$ is important for formic acid synthesis using the system shown in Scheme 2. Therefore, the optimum MV^{2+} concentration is 30 μM for photochemical formic acid synthesis with FDH. Next, let us focus on the concentration of an electron donating reagent, TEOA, on the initial rate and yield of $MV^{\bullet+}$ formation in the system containing ZnTMPyP, MV^{2+} and TEOA with visible light irradiation. At an MV^{2+} concentration of 30 μM, the MV^{2+} photoreduction rate was independent of TEOA concentration under pH 7 and 8. This result shows that the rate-limiting step of the MV^{2+} photoreduction is the electron transfer from *ZnTMPyP to MV^{2+}.

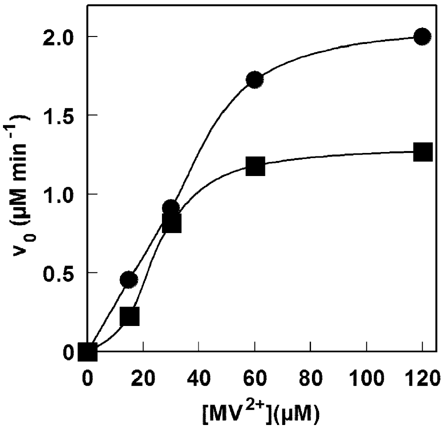


Fig. 1. The relationship between MV^{2+} concentration and the initial rate of the MV^{2+} photoreduction (v_0). Squares and circles are pH 7 and 8 conditions, respectively.

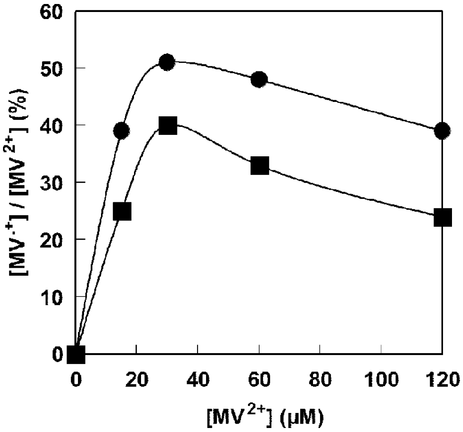


Fig. 2. The relationship between MV^{2+} concentration and the conversion yield of MV^{2+} to $MV^{\bullet+}$ ($[MV^{\bullet+}]/[MV^{2+}]$). Squares and circles are pH 7 and 8 conditions, respectively.

3.2. Formic acid synthesis from hydrogen carbonate ion with FDH and ZnTMPyP by visible light

Photochemical formic acid synthesis from hydrogen carbonate ion was investigated by adding FDH to the MV^{2+} photoreduction system using photosensitisation of ZnTMPyP in the presence of TEOA. When the reaction mixture containing ZnTMPyP, MV^{2+}, TEOA, sodium hydrogen carbonate and FDH was irradiated with visible light at 30 °C, formic acid and the decrease of hydrogen carbonate ion were observed with irradiation time. Therefore, visible light-induced conversion system of hydrogen carbonate ion to formic acid was achieved. Fig. 3 shows the relationship between the formic acid synthesis rate (v) and FDH activities. The symbols of squares and circles in Fig. 3 are pH 7 and 8 conditions, respectively. The formic acid synthesis rate was calculated by the concentration of formic acid after 1 h irradiation. Formic acid increased with FDH activity up to 20 units and then decreased. Therefore, the maximum

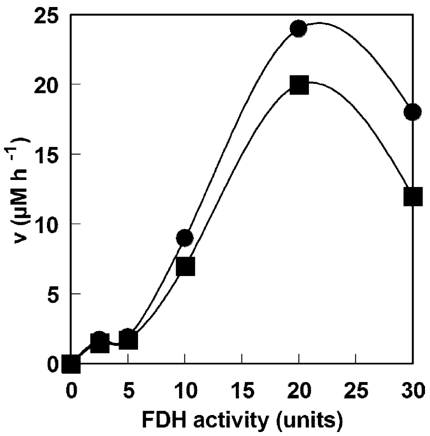


Fig. 3. The effect of FDH activities on the photochemical formic acid synthesis rate (v). Squares and circles are pH 7 and 8 conditions, respectively. The v is calculated from the formic acid concentration after 3 h irradiation.

activity of FDH is 20 units for photochemical formic acid synthesis. At 20 units FDH, the formic acid synthesis rates under pH 7 and 8 were 19.8 and 24.0 μM h^{-1}, respectively. Under the amount of FDH over 20 units, the concentration of $MV^{\bullet+}$ will be run short to the amount of FDH and the oxidation of formic acid to hydrogen carbonate ion with excess MV^{2+} and FDH catalytic reaction will proceed. Thus, the formic acid synthesis rate decreases under over 20 units FDH conditions. In contrast, FDH will be run short to the concentration of $MV^{\bullet+}$ under the amount of FDH up to 20 units. Thus, the formic acid synthesis rate increased with increasing FDH units. From the results of Fig. 3, the photochemical formic acid synthesis was also promoted under high pH condition. Next, let us focus on the effect of MV^{2+} concentration on the photochemical formic acid synthesis in the system containing ZnTMPyP, MV^{2+}, TEOA, sodium hydrogen carbonate and FDH with visible light irradiation. Fig. 4 shows the relationship between the formic acid synthesis rate and MV^{2+} concentrations. The symbols of squares and circles in Fig. 4 are pH 7 and 8 conditions, respectively. The formic acid synthesis rate increased with the concentration of MV^{2+} up to 30 μM and then gradually decreased. The formic acid synthesis rates under pH 7 and 8 were 19.8 and 24.0 μM h^{-1}, respectively. At 20 units FDH and 30 μM MV^{2+}, the concentration of formic acid under pH 7 and 8 condition were 50 and 62 μM after 3 h irradiation, respectively. On the other hand, no formic acid was produced in the above system in the absence of FDH. Therefore, hydrogen carbonate ion was not directly reduced to formic acid by $MV^{\bullet+}$. The activity changes of FDH under pH 7 and 8 is tested in the reaction system containing 1.0 mM NADH, 20 units FDH and 10 mM sodium hydrogen carbonate at 30 °C. When the reaction mixture was incubated for 1 h, the concentrations of formic acid produced under pH 7 and 8 were 20 and 31 μM, respectively. The FDH activity increased under higher pH value. In addition, the MV^{2+} photoreduction rate also increased under higher pH value. Thus, the overall reaction rate increased under higher pH condition. The activity changes of FDH after 3 h irradiation is tested in the reaction system containing 1.0 mM NADH, 20 units FDH and 10 mM sodium hydrogen carbonate at 30 °C. The 20 units FDH which irradiated light beforehand for 3 h was added to the reaction solution containing 1.0 mM NADH and 10 mM sodium hydrogen carbonate, and was then incubated for 1 h. The concentration of formic acid produced was 80 μM. In contrast, the 20 units FDH without irradiation was added to reaction solution containing 1.0 mM NADH and 10 mM sodium hydrogen carbonate, and was then incubated for 1 h. The concentration of formic acid produced was 85 μM. By 3 h irradiation, the FDH activity slightly decreased. However, most activities of FDH were not lost. Fig. 5 shows the time dependence of photochemical formic acid synthesis with

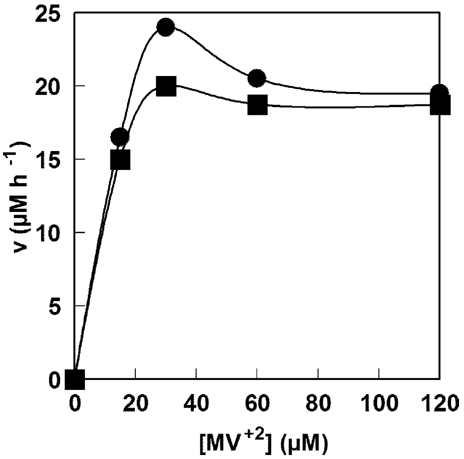


Fig. 4. The relationship between MV^{2+} concentration and the photochemical formic acid synthesis rate (v). Squares and circles are pH 7 and 8 conditions, respectively. The v is calculated from the formic acid concentration after 1 h irradiation.

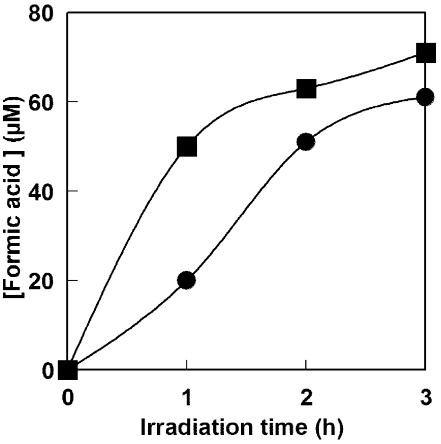


Fig. 5. Time dependence of photochemical formic acid synthesis under steady state irradiation with visible light using a 200 W tungsten lamp at a distance of 3.0 cm. The solution contained TEOA (0.3 M), ZnTMPyP, MV^{2+} (30 μM), $NaHCO_3$ (1.0 mM) and FDH in 3.0 ml of 10 mM^3 potassium phosphate buffer (pH 8.0). Squares and circles are 90 and 9.0 μM ZnTMPyP, respectively. The reaction temperature was 30 °C.

the system containing ZnTMPyP, TEOA, MV^{2+}, sodium hydrogen carbonate and FDH at pH 8, under continuous irradiation at 30 °C. The symbols of squares and circles are 90 and 9.0 μM ZnTMPyP, respectively. The initial rate of formic acid synthesis increased with increasing ZnTMPyP concentration. However little change in the concentration of formic acid after 3 h irradiation was observed by the increasing ZnTMPyP concentration. These results show that photochemical formic acid synthesis was achieved with FDH and $MV^{\bullet+}$ produced by the photosensitisation of ZnTMPyP in the presence of TEOA.

4. Conclusion

In this work, photochemical and enzymatic formic acid synthesis from hydrogen carbonate ion with FDH from *Saccharomyces cerevisiae* and $MV^{\bullet+}$ produced by the visible light photosensitisation of ZnTMPyP is developed. For MV^{2+} photoreduction, the initial rate increased with the concentration of MV^{2+}. However, the yield of MV^{2+} to $MV^{\bullet+}$ increased with the concentration of MV^{2+} up to 30 μM and then gradually decreased. Photochemical formic acid synthesis from hydrogen carbonate ion was investigated by adding FDH to the MV^{2+} photoreduction system using photosensitisation of ZnTMPyP in the presence of TEOA and formic acid, and decrease of hydrogen carbonate ion were observed with irradiation time. Formic acid increased with FDH activity up to 20 units and then decreased and the formic acid synthesis rate under pH 8 was 24.0 μM h^{-1} at 20 units FDH. For photochemical formic acid synthesis, the optimum MV^{2+} concentration is 30 μM and the concentration of formic acid under pH 8 condition was 62 μM after 3 h irradiation. This system is formic acid from hydrogen carbonate ion with FDH in the absence of a natural cofactor NADH and an enzyme FDR. This system could be developed to convert CO_2 into useful organic acid materials. Now we are attempting the up-scaling reaction and photochemical formic acid synthesis using sunlight for the development of economic processing of formic acid.

Acknowledgements

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