Visible Light-induced Formic Acid Synthesis from HCO$_3^-$ with Formate Dehydrogenase and Water-soluble Zinc Porphyrin

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Photochemical and enzymatic synthesis of formic acid was investigated from HCO$_3^-$ with formate dehydrogenase (FDH) from *Saccharomyces cerevisiae* and reduced methylviologen (MV$^{2+}$) produced by the visible light photosensitization of zinc tetraakis(4-methylpyridyl) porphyrin (ZnTMPyP) in the presence of triethanolamine (TEOA) as an electron donating reagent. Irradiation of a solution containing TEOA, ZnTMPyP, MV$^{2+}$, NaHCO$_3$ and FDH in potassium phosphate buffer, with visible light resulted in formic acid production and HCO$_3^-$ consumption. The optimum FDH activity was 20 units and the amount of formic acid and the yield of HCO$_3^-$ to formic acid after 4 h irradiation were estimated to be 50 µmol·dm$^{-3}$ and 5.0%, respectively.

Keywords
Formic acid synthesis, Zinc porphyrin, Formate dehydrogenase, Enzymatic synthesis

1. Introduction

Carbon dioxide fixation is a potential technology for the realization of photocatalytic CO$_2$ reduction. Many studies on CO$_2$ fixation have investigated photocatalysis on semiconductors such as titanium dioxide, silicon carbide$^1$ and strontium titanate$^2)$. However, these systems used ultraviolet irradiation, whereas a CO$_2$ fixation system using visible light is more desirable. Visible light-induced photoredox systems consisting of an electron donor, a photosensitizer, and an electron relay, have been widely studied$^3)$. The photosensitizer is an essential component of such systems. The photoexcited triplet state of the photosensitizer is important, because the triplet state has a longer lifetime than the photoexcited singlet state.

Ruthenium(II) coordination compounds and porphyrins are widely used as effective photosensitizers in photoredox systems. Ruthenium(II) coordination compounds and porphyrins have different photophysical properties as follows. The lifetime of the photoexcited triplet state and the peak of the absorption band with maximum wavelength are 0.65 ms and 452 nm, respectively, for tris(bipyridinium) ruthenium(II) (Ru(bpy)$_3^{2+}$), but are 1500 ms and 1000 ms, respectively, for zinc porphyrins, zinc tetraphenylporphyrin tetratsulfonate (ZnTPPS) and zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP). The porphyrins have strong absorption bands in the visible light region (380-600 nm). Therefore, these porphyrins are more widely used as an effective photosensitizer than Ru(bpy)$_3^{2+}$$^4)$. In particular, the water-soluble zinc porphyrins, ZnTPPS and ZnTMPyP, are useful as photosensitizers$^7,8)$. Biological CO$_2$ fixation systems have also received much attention. For example, CO$_2$ or HCO$_3^-$ can be reduced to formic acid with formate dehydrogenase (FDH) and NADP$^+$.$^9$ Therefore, a CO$_2$ fixation system that combines the photoreduction of NAD$^+$ by the photosensitization of zinc porphyrin and ferredoxin-NADP$^+$ reductase, and HCO$_3^-$ reduction with FDH as shown in Scheme 1 a) can be established. The CO$_2$ absorption bands in the visible light region (380-600 nm). Therefore, these porphyrins are more widely used as an effective photosensitizer than Ru(bpy)$_3^{2+}$.$^4)$. In particular, the water-soluble zinc porphyrins, ZnTPPS and ZnTMPyP, are useful as photosensitizers$^7,8)$. Biological CO$_2$ fixation systems have also received much attention. For example, CO$_2$ or HCO$_3^-$ can be reduced to formic acid with formate dehydrogenase (FDH) and NADP$^+$.$^9$ Therefore, a CO$_2$ fixation system that combines the photoreduction of NAD$^+$ by the photosensitization of zinc porphyrin and ferredoxin-NADP$^+$ reductase, and HCO$_3^-$ reduction with FDH as shown in Scheme 1 a) can be established. The CO$_2$
fixation system based on malic acid synthesis combined with the photoreduction of NAD\(^+\) by the photosensitization of chemical-modified chlorophyll and ferredoxin-NAD\(^+\) reductase, and malic acid synthesis from pyruvate and \(\text{HCO}_3^-\) with malate dehydrogenase enzyme has also been reported \(^{10}\).

Our investigation first attempted the development of the visible light-induced formic acid synthesis from \(\text{HCO}_3^-\) as shown in Scheme 1 a). However, the conversion yield of NAD\(^+\) to NADH with ferredoxin-NAD\(^+\) reductase was very low. Moreover, ferredoxin-NAD\(^+\) reductase is very expensive. If methylviologen (MV\(^{2+}\)), the cheaper electron mediator, can be used instead of NAD\(^+\), an effective CO\(_2\) fixation scheme could be established without requiring ferredoxin-NAD\(^+\) reductase and NAD\(^+\) as shown in Scheme 1 b). Enzymatic formic acid synthesis from \(\text{HCO}_3^-\) with FDH and MV\(^{2+}\) photoreduction with a system containing Ru(bpy)\(_3\)\(^2+\) as a photosensitizer and mercaptoethanol (RSH) as an electron donor has been reported \(^{11}\). The one-electron oxidized form of RSH is the thioether (RS-SR) and RS-SR is irreversibly reduced to RSH by donation of a single electron. Therefore, the formic acid synthesis will stop after complete conversion of RSH to RS-SR. In contrast, triethanolamine (TEOA) is a good electron donating reagent and widely used in photoredox systems as a sacrificial reagent. The one-electron oxidized form of TEOA (TEOA\(_{\text{ox}}\)) can be regenerated to TEOA by one-electron reduction. If the TEOA regeneration system is substituted in the reaction system shown in Scheme 1 b), continuous formic acid synthesis could be established.

Here we describe a system for visible light-induced formic acid synthesis from \(\text{HCO}_3^-\) with formate dehydrogenase (FDH) from \(\text{Saccharomyces cerevisiae}\) and tetraethylammonium chloride 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 tetrathylammonium 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 (Sepahdex LH-20, eluant: water).

2.3. Photoreduction of MV\(^{2+}\) by Photosensitization of ZnTMPyP

A solution containing ZnTMPyP (9.0 \(\mu\text{mol} \cdot \text{dm}^{-3}\)), MV\(^{2+}\) and TEOA (0.3 \(\mu\text{mol} \cdot \text{dm}^{-3}\)) as an electron-donating reagent in 3.0 \(\text{ml}\) of 10 \(\mu\text{mol} \cdot \text{dm}^{-3}\) potassium phosphate buffer (pH 7.0) was de aerated by freeze-pump-thaw cycles repeated 6 times. The sample solution was irradiated with a 200 W tungsten lamp (Philips) at a distance of 3.0 cm. To prevent degradation of ZnTMPyP by near UV light, wavelengths of less than 390 nm were blocked with a Toshiba L-39 cut-off filter. The light intensity was measured by potassium ferrioxalate actinometry \(^{13}\). Reduced MV\(^{2+}\) (MV\(^{-}\)) concentration was determined by the absorbance at 605 nm using the molar coefficient 1.3 \(\times\) 10\(^4\) \(\text{mol}^{-1} \cdot \text{dm}^{-3} \cdot \text{cm}^{-1}\) \(^{14}\). MV\(^{2+}\) concentrations in the solution were varied between 15 \(\mu\text{mol} \cdot \text{dm}^{-3}\) and 120 \(\mu\text{mol} \cdot \text{dm}^{-3}\).

2.4. Visible Light-induced Formic Acid Synthesis from \(\text{HCO}_3^-\) with FDH and ZnTMPyP

A solution containing ZnTMPyP (9.0 \(\mu\text{mol} \cdot \text{dm}^{-3}\)), MV\(^{2+}\) (15 \(\mu\text{mol} \cdot \text{dm}^{-3}\)), TEOA (0.3 \(\mu\text{mol} \cdot \text{dm}^{-3}\)) and FDH was de aerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 5 min. NaHCO\(_3\) solution (1 \(\mu\text{mol} \cdot \text{dm}^{-3}\)) 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. The amounts of formic acid and \(\text{HCO}_3^-\) were detected by HPLC with an electrical conductivity detector (Shimadzu CDD-10AP) (column temperature: 40°C, column: polysulphene sulfonate column Shimadzu SCR-H, eluant: \(p\)-toluene sulfonic acid, flow rate: 0.8 \text{ml} \cdot \text{min}^{-1}\)). FDH
activities in a solution were varied between 2.5 units and 30 units.

3. Results and Discussion

3.1. Photoreduction of MV²⁺ with Visible Light Sensitization of ZnTMPyP

The time dependence of the MV⁺⁺ concentration in the system containing ZnTMPyP, MV²⁺ and TEOA under visible light irradiation is shown in Fig. 1. The absorbance at 605 nm attributed to the absorption band of MV⁺⁺ increased with irradiation time. The initial rate of MV⁺⁺ formation was determined by the MV⁺⁺ concentration after irradiation for 10 min. The initial rate, the amount of MV⁺⁺ produced, the conversion yield of MV²⁺ to MV⁺ and the quantum yield by potassium ferrioxalate actinometry after 40 min irradiation under various MV²⁺ concentrations are summarized in Table 1. The rate of formation increased with the concentration of MV²⁺. In contrast, the conversion yield of MV²⁺ to MV⁺ increased with the concentration of MV²⁺ up to 30 μmol·dm⁻³ and then decreased. FDH catalyzes both the reduction of HCO₃⁻ to formic acid and the oxidation of formic acid to HCO₂⁻. The reaction rate of the reduction of HCO₃⁻ to formic acid is 20 times slower than that of the oxidation of formic acid to HCO₂⁻ in the system with FDH. At high MV²⁺ concentrations, HCO₂⁻ was produced by the oxidation of formic acid with FDH. The conversion yield of MV²⁺ to MV⁺ is important for formic acid synthesis using the system shown in Scheme 1b. Therefore, the optimum MV²⁺ concentration is 30 mmol·dm⁻³ for formic acid synthesis with FDH.

The effect of TEOA concentration on the time dependence of MV⁺⁺ formation in the system containing ZnTMPyP, MV²⁺ and TEOA with visible light irradiation is shown in Fig. 2. At a MV²⁺ concentration of 30 μmol·dm⁻³, the MV²⁺ photoreduction rate was independent of TEOA concentration. This result shows that the electron transfer from TEOA to the one-electron oxidized ZnTMPyP (ZnTMPyP⁺) is faster than the electron transfer from the photoexcited ZnTMPyP (*ZnTMPyP) to MV²⁺ in the MV²⁺ photoreduction process. Therefore, the rate-limiting step of the MV²⁺ photoreduction is the electron transfer from *ZnTMPyP to MV²⁺.

3.2. Formic Acid Synthesis from HCO₃⁻ with FDH and ZnTMPyP by Visible Light

Formic acid synthesis from HCO₃⁻ was investigated by adding FDH to the MV²⁺ photoreduction system using photosensitization of ZnTMPyP in the presence of TEOA. The time dependence of the formic acid synthesis in the system containing TEOA, ZnTMPyP, MV²⁺, NaHCO₃ and FDH in potassium phosphate buffer (pH 7.0) is shown in Fig. 3(a). The time dependence of HCO₃⁻ in this system is shown in Fig. 3(b). HCO₃⁻ decreased with increased formic acid production under continuous irradiation. Therefore, visible light-induced conversion system of HCO₃⁻ to formic acid was achieved. The formic acid produced and quantum yield by potassium ferrioxalate actinometry after 40 min irradiation at various FDH activities are summarized in Table 2. Formic acid formation increased with FDH activity up to 20 units and then decreased. Formic acid formation under the above conditions was 50 μmol·dm⁻³ and the conversion yield of HCO₃⁻ to formic acid was about 5.0% after 4 h irra-

Table 1 Initial Rate (v₀), Amount of MV⁺⁺ produced ([MV⁺⁺]), Conversion Yield of MV⁺⁺ to MV⁺ ([MV⁺⁺]/[MV⁺⁺]) and Quantum Yield (φ) at Various MV²⁺ Concentrations

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<th>[MV²⁺] [μmol·dm⁻³]</th>
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<th>[MV⁺⁺] [μmol·dm⁻³]</th>
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The solution contained TEOA (0.3 mol·dm⁻³), ZnTMPyP (9.0 μmol·dm⁻³) and MV²⁺ in 3.0 ml of 10 mmol·dm⁻³ potassium phosphate buffer (pH 7.0). Concentrations of MV²⁺ are (■): 15, (△): 30, (▲): 60 and (●): 120 μmol·dm⁻³, respectively.

Fig. 1 Time Dependence of MV²⁺ Reduction under Steady State Irradiation with Visible Light Using a 200 W Tungsten Lamp at a Distance of 3.0 cm

The yield of MV²⁺ under visible light irradiation is shown in the system containing ZnTMPyP, MV²⁺ and TEOA with visible light irradiation is shown in Fig. 1. The absorbance at 605 nm attributed to the absorption band of MV⁺⁺ increased with irradiation time. The initial rate of MV⁺⁺ formation was determined by the MV⁺⁺ concentration after irradiation for 10 min. The initial rate, the amount of MV⁺⁺ produced, the conversion yield of MV²⁺ to MV⁺ and the quantum yield by potassium ferrioxalate actinometry after 40 min irradiation under various MV²⁺ concentrations are summarized in Table 1. The rate of formation increased with the concentration of MV²⁺. In contrast, the conversion yield of MV²⁺ to MV⁺ increased with the concentration of MV²⁺ up to 30 μmol·dm⁻³ and then decreased. FDH catalyzes both the reduction of HCO₃⁻ to formic acid and the oxidation of formic acid to HCO₂⁻. The reaction rate of the reduction of HCO₃⁻ to formic acid is 20 times slower than that of the oxidation of formic acid to HCO₂⁻ in the system with FDH. At high MV²⁺ concentrations, HCO₂⁻ was produced by the oxidation of formic acid with FDH. The conversion yield of MV²⁺ to MV⁺ is important for formic acid synthesis using the system shown in Scheme 1b. Therefore, the optimum MV²⁺ concentration is 30 mmol·dm⁻³ for formic acid synthesis with FDH.

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diation with 20 units of FDH. The turnover number of MV^{2+} was estimated to be 0.03 min^{-1}. On the other hand, no formic acid was produced in the above system in the absence of FDH. Therefore, HCO_3^{-} was not directly reduced to formic acid by MV·. In contrast, formic acid synthesis was reported from HCO_3^{-} in a system containing FDH, MV^{2+}, Ru(bpy)_3^{2+} and RSH, and the turnover number of MV^{2+} was estimated to be 0.01 min^{-1}. Thus, effective formic acid synthesis was achieved with FDH and MV· produced by the photosensitization of ZnTMPyP in the presence of TEOA.

4. Conclusion

Photochemical and enzymatic CO_2 fixation based on formic acid synthesis from HCO_3^{-} with FDH from *Saccharomyces cerevisiae* and MV· produced by the visible light photosensitization of ZnTMPyP can continuously synthesize formic acid. This system could be developed to convert CO_2 into useful organic acid materials. We are now investigating visible light-induced and enzymatic methanol synthesis from CO_2 using the above system and alcohol dehydrogenase.

Acknowledgment

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<th>FDH [units]</th>
<th>[HCOOH] [µmol·dm^{-3}]</th>
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<th>φ [%]</th>
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The solution contained TEOA, ZnTMPyP (9.0 µmol·dm^{-3}) and MV^{2+} (30 µmol·dm^{-3}) in 3.0 ml of 10 mmol·dm^{-3} potassium phosphate buffer (pH 7.0). Concentrations of TEOA are (■): 0.1, (□): 0.3, and (○): 1.0 mol·dm^{-3}, respectively.

Fig. 2 Time Dependence of MV^{2+} Reduction 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 mol·dm^{-3}), ZnTMPyP (9.0 µmol·dm^{-3}), MV^{2+} (30 µmol·dm^{-3}), NaHCO_3 (1.0 mmol·dm^{-3}) and FDH in 3.0 ml of 10 mmol·dm^{-3} potassium phosphate buffer (pH = 7.0). The activities of FDH are (□): 0, (■): 5, (○): 10, (▲): 15, (●): 20, (○): 30 units, respectively.

Fig. 3 Time Dependence of Formic Acid Production (a) and HCO_3^{-} Consumption (b) under Steady State Irradiation with Visible Light Using a 200 W Tungsten Lamp at a Distance of 3.0 cm
References


要 旨

ギ酸脱水素酵素と亜鉛ボルフィリンを用いた可視光による炭酸水素イオンからのギ酸合成反応

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