

[Regular Paper]

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 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+} , 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 $50 \mu\text{mol} \cdot \text{dm}^{-3}$ and 5.0%, respectively.

Keywords

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

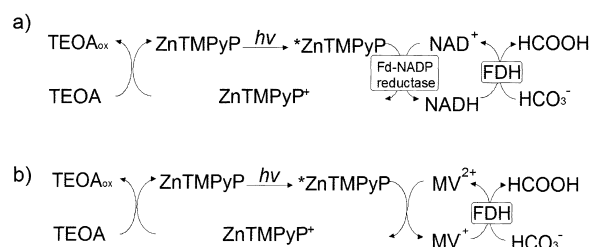
1. Introduction

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 carbide¹⁾ and strontium titanate²⁾. 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 studied³⁾. 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) ($\text{Ru}(\text{bpy})_3^{2+}$), but are 1500 ms and 1000 ms, respectively, for zinc porphyrins, zinc tetraphenylporphyrin tetrasulfonate (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 $\text{Ru}(\text{bpy})_3^{2+}$ ^{4)–6)}. 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 $\text{NADH}^9)$. 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



a) Formic acid synthesis from HCO_3^- with formate dehydrogenase (FDH) and the photoreduction of NAD^+ with TEOA, ZnTMPyP, and ferredoxin-NADP⁺ reductase (Fd-NADP reductase).

b) Formic acid synthesis from HCO_3^- with FDH and the photoreduction of MV^{2+} with TEOA and ZnTMPyP.

Scheme 1

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fixation system based on malic acid synthesis combined with the photoreduction of NAD^+ by the photosensitization of chemical-modified chlorophyll and ferredoxin-NADP⁺ reductase, and malic acid synthesis from pyruvate and HCO_3^- with malate dehydrogenase enzyme has also been reported¹⁰.

Our investigation first attempted the development of the visible light-induced formic acid synthesis from HCO_3^- as shown in **Scheme 1 a**). However, the conversion yield of NAD^+ to NADH with ferredoxin-NADP⁺ reductase was very low. Moreover, ferredoxin-NADP⁺ reductase is very expensive. If methylviologen (MV^{2+}), the cheaper electron mediator, can be used instead of NAD^+ , an effective CO_2 fixation system could be established without requiring ferredoxin-NADP⁺ reductase and NAD^+ as shown in **Scheme 1 b**). Enzymatic formic acid synthesis from HCO_3^- with FDH and MV^{2+} photoreduction with a system containing $\text{Ru}(\text{bpy})_3^{2+}$ as a photosensitizer and mercaptoethanol (RSH) as an electron donor has been reported¹¹. 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_{ox}) can be regenerated to TEOA by one-electron donation. 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 HCO_3^- with formate dehydrogenase (FDH) from *Saccharomyces cerevisiae* and reduced MV^{2+} produced by the visible light photosensitization of ZnTMPyP in the presence of TEOA as an electron donor.

2. Experimental

2.1. Materials

FDH from *Saccharomyces cerevisiae* was obtained from Sigma Co., Ltd. Methyl viologen dichloride (MV^{2+}), tetrakis(4-methylpyridyl) porphyrin (TMPyP) and triethanolamine (TEOA) 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 reduce 1.0 mmol formic acid to HCO_3^- per min in the presence of NAD^+ .

2.2. Preparation of Zinc Tetrakis(4-methylpyridyl) Porphyrin (ZnTMPyP)

Zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP) 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 h¹²). 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, elutant: 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 ($0.3 \text{ mol} \cdot \text{dm}^{-3}$) as an electron-donating reagent in 3.0 ml of $10 \text{ mmol} \cdot \text{dm}^{-3}$ potassium phosphate buffer (pH 7.0) was deaerated 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 actinometry¹³. Reduced MV^{2+} ($\text{MV}^{\cdot+}$) 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 HCO_3^- with FDH and ZnTMPyP

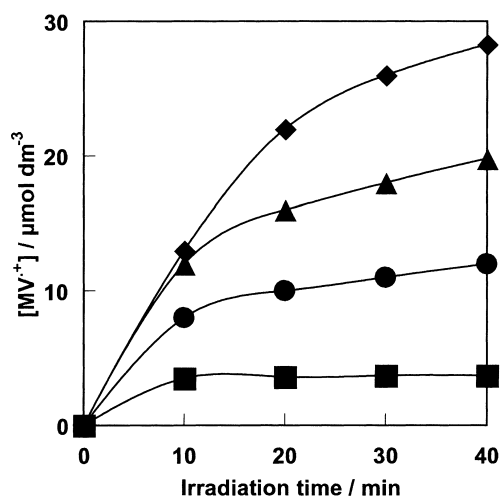
A solution containing ZnTMPyP ($9.0 \mu\text{mol} \cdot \text{dm}^{-3}$), MV^{2+} ($15 \mu\text{mol} \cdot \text{dm}^{-3}$), TEOA ($0.3 \text{ mol} \cdot \text{dm}^{-3}$) and FDH was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 5 min. NaHCO_3 solution ($1 \text{ mmol} \cdot \text{dm}^{-3}$) 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 HCO_3^- 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, 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 MV^{2+} with Visible Light Sensitization of ZnTMPyP

The time dependence of the $MV^{\cdot+}$ concentration in the system containing ZnTMPyP, MV^{2+} and TEOA under visible light irradiation is shown in **Fig. 1**. The absorbance at 605 nm attributed to the absorption band of $MV^{\cdot+}$ increased with irradiation time. The initial rate of $MV^{\cdot+}$ formation was determined by the $MV^{\cdot+}$ concentration after irradiation for 10 min. The initial rate, the amount of $MV^{\cdot+}$ produced, the conversion yield of MV^{2+} to $MV^{\cdot+}$ and the quantum yield by potassium ferrioxalate actinometry after 40 min irradiation under various MV^{2+} concentrations are summarized in **Table 1**. The rate of formation increased with the concentration of MV^{2+} . In contrast, the conversion yield of MV^{2+} to $MV^{\cdot+}$ increased with the concentration



The solution contained TEOA ($0.3 \text{ mol} \cdot \text{dm}^{-3}$), ZnTMPyP ($9.0 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$) and MV^{2+} in 3.0 ml of $10 \text{ mmol} \cdot \text{dm}^{-3}$ potassium phosphate buffer (pH 7.0). Concentrations of MV^{2+} are (■): 15, (●): 30, (▲): 60 and (◆): $120 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$, respectively.

Fig. 1 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

Table 1 Initial Rate (v_0), Amount of $MV^{\cdot+}$ produced ($[MV^{\cdot+}]$), Conversion Yield of MV^{2+} to $MV^{\cdot+}$ ($[MV^{\cdot+}]/[MV^{2+}]$) and Quantum Yield (ϕ) at Various MV^{2+} Concentrations

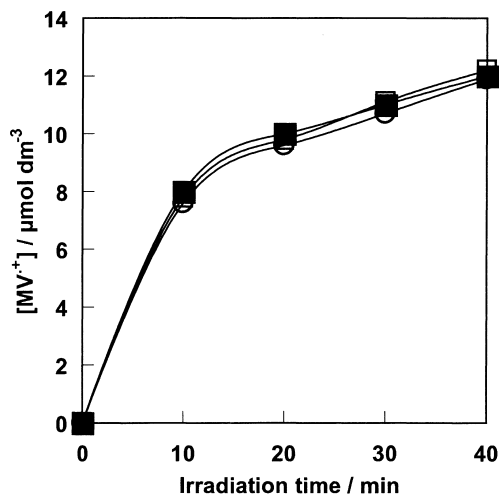
| $[MV^{2+}]$ $[\mu\text{mol} \cdot \text{dm}^{-3}]$ | v_0 $[\mu\text{mol} \cdot \text{dm}^{-3} \cdot \text{min}^{-1}]$ | $[MV^{\cdot+}]$ $[\mu\text{mol} \cdot \text{dm}^{-3}]$ | $[MV^{\cdot+}]/[MV^{2+}]$ [%] | ϕ [%] |
|---|---|---|----------------------------------|---------------|
| 15 | 0.13 | 3.7 | 25 | 0.07 |
| 30 | 0.8 | 12 | 40 | 0.2 |
| 60 | 1.2 | 20 | 33 | 0.36 |
| 120 | 1.3 | 28 | 24 | 0.51 |

of MV^{2+} up to $30 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$ and then decreased. FDH catalyzes both the reduction of HCO_3^- to formic acid and the oxidation of formic acid to HCO_3^- . The reaction rate of the reduction of HCO_3^- to formic acid is 20 times slower than that of the oxidation of formic acid to HCO_3^- in the system with FDH. At high MV^{2+} concentrations, HCO_3^- was produced by the oxidation of formic acid with FDH. The conversion yield of MV^{2+} to $MV^{\cdot+}$ is important for formic acid synthesis using the system shown in **Scheme 1 b**). Therefore, the optimum MV^{2+} concentration is $30 \text{ mmol} \cdot \text{dm}^{-3}$ for formic acid synthesis with FDH.

The effect of TEOA concentration on the time dependence of $MV^{\cdot+}$ formation in the system containing ZnTMPyP, MV^{2+} and TEOA with visible light irradiation is shown in **Fig. 2**. At a MV^{2+} concentration of $30 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$, the MV^{2+} 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 ($*\text{ZnTMPyP}$) to MV^{2+} in the MV^{2+} photoreduction process. Therefore, the rate-limiting step of the MV^{2+} photoreduction is the electron transfer from $*\text{ZnTMPyP}$ to MV^{2+} .

3.2. Formic Acid Synthesis from HCO_3^- with FDH and ZnTMPyP by Visible Light

Formic acid synthesis from HCO_3^- was investigated by adding FDH to the MV^{2+} 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, MV^{2+} , NaHCO_3 and FDH in potassium phosphate buffer (pH 7.0) is shown in **Fig. 3(a)**. The time dependence of HCO_3^- in this system is shown in **Fig. 3(b)**. HCO_3^- decreased with increased formic acid production under continuous irradiation. Therefore, visible light-induced conversion system of HCO_3^- 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 $50 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$ and the conversion yield of HCO_3^- to formic acid was about 5.0% after 4 h irra-



The solution contained TEOA, ZnTMPyP ($9.0 \mu\text{mol} \cdot \text{dm}^{-3}$) and MV^{2+} ($30 \mu\text{mol} \cdot \text{dm}^{-3}$) in 3.0 ml of $10 \text{ mmol} \cdot \text{dm}^{-3}$ potassium phosphate buffer (pH 7.0). Concentrations of TEOA are (■): 0.1, (□): 0.3, and (○): $1.0 \text{ mol} \cdot \text{dm}^{-3}$, 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

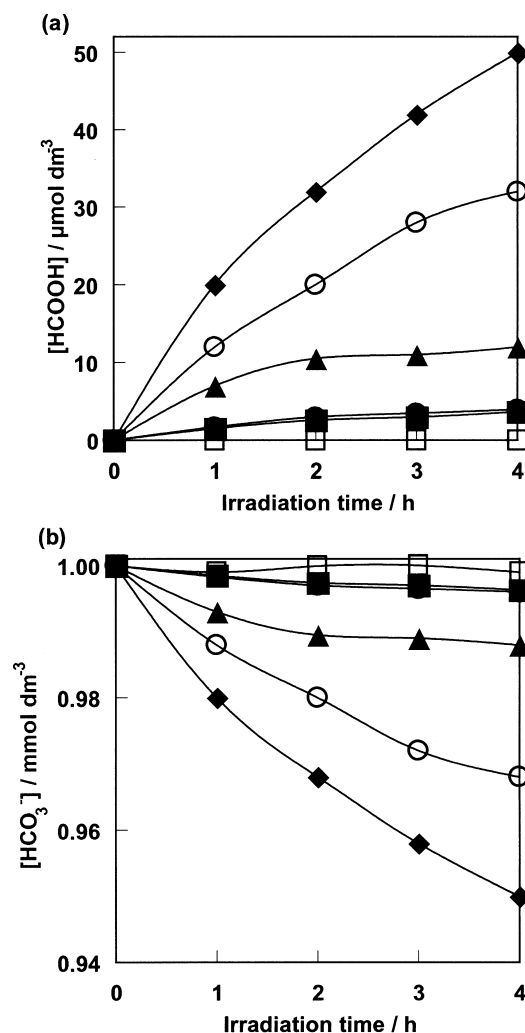
irradiation with 20 units of FDH. The turnover number of MV^{2+} was estimated to be 0.03 min^{-1} . On the other hand, no formic acid was produced in the above system in the absence of FDH. Therefore, HCO_3^- was not directly reduced to formic acid by $\text{MV}^{\cdot+}$. In contrast, formic acid synthesis was reported from HCO_3^- in a system containing FDH, MV^{2+} , $\text{Ru}(\text{bpy})_3^{2+}$ and RSH, and the turnover number of MV^{2+} was estimated to be 0.01 min^{-1} ¹¹⁾. Thus, effective formic acid synthesis from HCO_3^- can be developed using a system with ZnTMPyP and TEOA. These results show that formic acid synthesis was achieved with FDH and $\text{MV}^{\cdot+}$ 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 $\text{MV}^{\cdot+}$ 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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The solution contained TEOA ($0.3 \text{ mol} \cdot \text{dm}^{-3}$), ZnTMPyP ($9.0 \mu\text{mol} \cdot \text{dm}^{-3}$), MV^{2+} ($30 \mu\text{mol} \cdot \text{dm}^{-3}$), NaHCO_3 ($1.0 \text{ mmol} \cdot \text{dm}^{-3}$) and FDH in 3.0 ml of $10 \text{ mmol} \cdot \text{dm}^{-3}$ 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

Table 2 Formation of Formic Acid ($[\text{HCOOH}]$), Conversion Yield of HCO_3^- to HCOOH ($[\text{HCO}_3^-]/[\text{HCOOH}]$) and Quantum Yield (ϕ) at Various FDH Activities

| FDH [units] | $[\text{HCOOH}]$ [$\mu\text{mol} \cdot \text{dm}^{-3}$] | $[\text{HCO}_3^-]/[\text{HCOOH}]$ [%] | ϕ [%] |
|-------------|---|---------------------------------------|------------|
| 0 | 0 | 0 | 0 |
| 2.5 | 3.7 | 0.37 | 0.07 |
| 5 | 4.0 | 0.40 | 0.07 |
| 10 | 12 | 1.2 | 0.22 |
| 20 | 50 | 5.0 | 0.91 |
| 30 | 32 | 3.2 | 0.58 |

References

- 1) Inoue, T., Fujishima, A., Konishi, S., Honda, K., *Nature*, **277**, 637 (1979).
- 2) Aurian-Blajeni, B., Halmann, M., Manassen, J., *Solar Energy*, **25**, 165 (1980).
- 3) Darwent, J. R., Douglas, P., Harriman, A., Porter, G., Richoux, M. C., *Corrd. Chem. Rev.*, **44**, 93 (1982).
- 4) Okura, I., *Corrd. Chem. Rev.*, **68**, 53 (1985).
- 5) Amao, Y., Okura, I., *J. Mol. Catal. A: Chem.*, **103**, 69 (1995).
- 6) Amao, Y., Okura, I., *J. Mol. Catal. A: Chem.*, **105**, 125 (1996).
- 7) Okura, I., Aono, S., Yamada, A., *J. Phys. Chem.*, **89**, 1593 (1985).
- 8) Amao, Y., Okura, I., "Photocatalysis-Science and Technology," KODANSHA-Springer, (2002).
- 9) Gul-Karauler, N., *Biotechnol. Lett.*, **23**, 283 (2001).
- 10) Itoh, T., Asada, H., Tobioka, K., Koderu, Y., Matsushima, A., Hiroto, M., Nishimura, H., Kamachi, T., Okura, I., Inada, Y., *Bioconjugate Chem.*, **11**, 8 (2000).
- 11) Mandler, D., Witlner, I., *J. Chem. Soc. Perkin Trans II*, 997 (1988).
- 12) Amao, Y., Kamachi, T., Okura, I., *J. Photochem. Photobiol.*, **98**, 59 (1996).
- 13) Hatchard, C. G., Parker, C. A., *Proc. R. Soc.*, **A235**, 518 (1956).
- 14) Watanabe, T., Honda, K., *J. Phys. Chem.*, **86**, 2617 (1982).

要 旨

ギ酸脱水素酵素と亜鉛ポ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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ギ酸脱水素酵素による炭酸水素イオンのギ酸への変換反応と水溶性亜鉛ポルフィリンの光増感作用によるメチルピオローゲンの光還元反応とを組み合わせた可視光によるギ酸合成系を構築した。具体的には電子供与体であるトリエタノールアミン、亜鉛テトラキス(4-メチルピリジル)ポルフィリン、メチルピオローゲンおよびギ酸脱水素酵素からなる系である。この反応系に炭酸水素ナトリウムを添加し、200 W タングステンランプを

用い可視光を照射すると光照射時間とともに定常的にギ酸が生成した。また、ギ酸の生成とともに炭酸水素イオンの減少が観測された。ギ酸脱水素酵素の最適活性量は 20 units であり、このとき光照射 4 時間後のギ酸生成量は $50 \mu\text{mol} \cdot \text{dm}^{-3}$ であった。以上のことからギ酸脱水素酵素と亜鉛ポ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系の構築に成功した。