

## Photochemical and Enzymatic Synthesis of Methanol from $\text{HCO}_3^-$ with Dehydrogenases and Zinc Porphyrin

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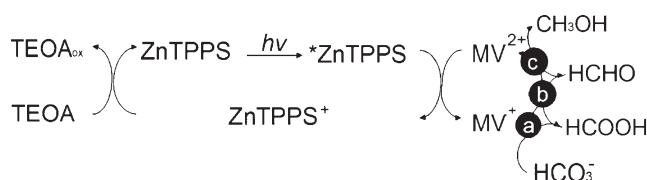
Photochemical and enzymatic methanol synthesis from  $\text{HCO}_3^-$  with formate dehydrogenase (FDH), aldehyde dehydrogenase (AldDH), and alcohol dehydrogenase (ADH) via the photoreduction of  $\text{MV}^{2+}$  using ZnTPPS photosensitization was developed and the amount of produced methanol was  $0.55 \mu\text{mol dm}^{-3}$  after 4-h irradiation.

$\text{CO}_2$  fixation is a potential technology for the realization of catalytic  $\text{CO}_2$  reduction.<sup>1,2</sup> Many studies on electrocatalysed  $\text{CO}_2$  reduction have been performed using specific electrode materials.<sup>3</sup> On the other hand, studies on  $\text{CO}_2$  fixation also have led to investigate photocatalysis on semiconductors such as titanium dioxide, silicon carbide, and strontium titanate.<sup>4,5</sup> However, these systems use ultraviolet irradiation and the total reaction is low yield, whereas highly efficient  $\text{CO}_2$  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.<sup>6</sup> The effective photosensitizer 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), have got a strong absorption bands in the visible light region (380–600 nm), these porphyrins are more widely used as an effective photosensitizer than ruthenium(II) coordination compounds.<sup>7–10</sup> On the other hand, biological and enzymatic  $\text{CO}_2$  fixation systems have also received much attention. For example,  $\text{CO}_2$  can be reduced to formic acid with formate dehydrogenase (FDH) and NADH.<sup>11</sup> Therefore, homogeneous photochemical  $\text{CO}_2$  reduction system that combines the photoreduction of  $\text{NAD}^+$  by the photosensitization of water-soluble zinc porphyrin and ferredoxin-NADP<sup>+</sup> reductase (FDR), and  $\text{CO}_2$  reduction with FDH can be established.

Some studies relating to photochemical  $\text{CO}_2$  reduction with enzyme have been reported. The  $\text{CO}_2$  fixation system based on malic acid synthesis combined with the photoreduction of  $\text{NAD}^+$  by the photosensitization of chemical-modified chlorophyll and FDR, and malic acid synthesis from pyruvate and  $\text{HCO}_3^-$  with malic enzyme has also been reported.<sup>12</sup> Enzymatic formic acid synthesis from  $\text{HCO}_3^-$  with FDH and  $\text{MV}^{2+}$  photoreduction with a system containing ruthenium(II) coordination compound as a photosensitizer and mercaptoethanol (RSH) as an electron donor also has been reported.<sup>13</sup> We previously reported the visible-light induced enzymatic formic acid synthesis from  $\text{HCO}_3^-$  with formate dehydrogenase (FDH) using the photosensitization of water-soluble zinc porphyrin.<sup>14–16</sup> A photochemical methanol synthesis from  $\text{HCO}_3^-$  system as shown in

Scheme 1 is accomplished by adding aldehyde dehydrogenase (AldDH) and alcohol dehydrogenase (ADH) in the above visible light-induced formic acid synthesis system. However, the photochemical and enzymatic methanol synthesis from  $\text{HCO}_3^-$  as shown in Scheme 1 with three dehydrogenases mentioned above and photosensitizer such as zinc porphyrin has not been developed yet.



**Scheme 1.** Photochemical and enzymatic methanol synthesis from  $\text{HCO}_3^-$  with FDH (a), AldDH (b), and ADH (c) via the photoreduction of  $\text{MV}^{2+}$  using ZnTPPS photosensitization.

In this letter, we describe a system for visible light-induced methanol synthesis from  $\text{HCO}_3^-$  with the system FDH, AldDH, and ADH, and  $\text{MV}^{2+}$  photoreduction by the visible light photosensitization of ZnTPPS in the presence of triethanol amine (TEOA) as an electron donor.

FDH and ADH were obtained from Sigma Co., Ltd. AldDH was purchased from Roche Co., Ltd. Tetraphenylporphyrin tetrasulfonate ( $\text{H}_2\text{TPPS}$ ) was purchased from Tokyo Chemical Industry 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  $1.0 \mu\text{mol}$  formic acid to  $\text{CO}_2$  per minute in the presence of  $\text{NAD}^+$ .

One unit of AldDH activity was defined as the amount of enzyme required to produce  $1.0 \mu\text{mol}$  formaldehyde to formic acid per minute in the presence of  $\text{NAD}^+$ .

One unit of ADH activity was defined as the amount of enzyme required to produce  $1.0 \mu\text{mol}$  methanol to formaldehyde per minute in the presence of  $\text{NAD}^+$ .

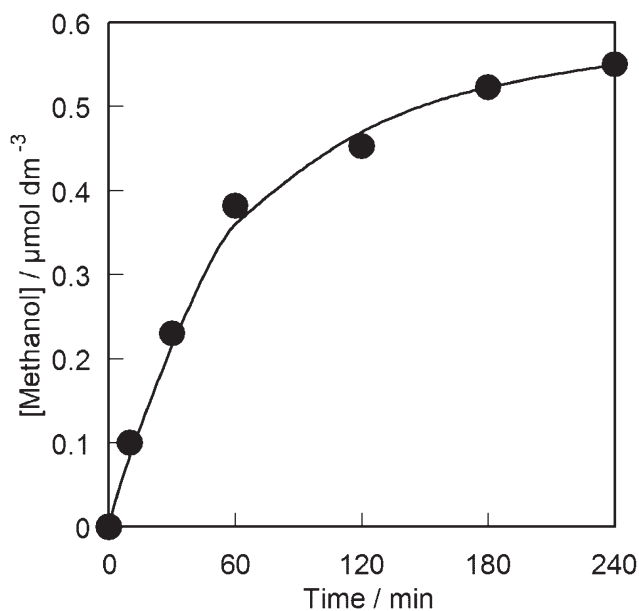
ZnTPPS was synthesized by refluxing  $\text{H}_2\text{TPPS}$  with excess molar of zinc acetate in methanol and then was evaporated to dryness in vacuo.<sup>17</sup>

Photoreduction of  $\text{MV}^{2+}$  is the most important step in photochemical synthesis of methanol from  $\text{HCO}_3^-$  system. To attain the highest yield of reduced  $\text{MV}^{2+}$  ( $\text{MV}^{\bullet+}$ ), the reaction conditions of photoreduction of  $\text{MV}^{2+}$  consisting of TEOA and ZnTPPS were investigated. A solution containing ZnTPPS ( $0.1 \mu\text{mol dm}^{-3}$ ),  $\text{MV}^{2+}$ , and TEOA ( $0.3 \text{ mol dm}^{-3}$ ) in  $3.0 \text{ mL}$  of  $10 \text{ mmol dm}^{-3}$  potassium phosphate buffer (pH 8) 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  $3.0 \text{ cm}$  with a Toshiba L-39 cut-off filter at

30 °C. The  $MV^{2+}$  concentration was determined by the absorbance at 605 nm using the molar coefficient  $13000 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ .<sup>18</sup>

When the sample solution was irradiated, the accumulation of  $MV^{2+}$  was observed. The rate of  $MV^{2+}$  formation increased with the concentration of  $MV^{2+}$ . In contrast, the conversion yield of  $MV^{2+}$  to  $MV^{+}$  increased with the concentration of  $MV^{2+}$  up to  $0.1 \text{ mmol dm}^{-3}$  and then decreased. FDH catalyzes both the reduction of  $\text{HCO}_3^-$  to formic acid and the oxidation of formic acid to  $\text{HCO}_3^-$ . The reaction rate of the reduction of  $\text{HCO}_3^-$  to formic acid is 20 times slower than that of the oxidation of formic acid to  $\text{HCO}_3^-$  in the system with FDH. At high  $MV^{2+}$  concentrations,  $\text{HCO}_3^-$  was produced by the oxidation of formic acid with FDH. The conversion yield of  $MV^{2+}$  to  $MV^{+}$  is important for formic acid synthesis using the system shown in Scheme 1. Therefore, the optimum  $MV^{2+}$  concentration is  $0.1 \text{ mmol dm}^{-3}$  for methanol synthesis. After 40 min irradiation,  $25 \mu\text{mol dm}^{-3}$   $MV^{2+}$  was produced. The reduction ratio of  $MV^{2+}$  to  $MV^{+}$  was about 25% after 40 min irradiation.

As the  $MV^{2+}$  photoreduction system with the photosensitization of ZnTPPS was developed, the photochemical methanol synthesis from  $\text{HCO}_3^-$  was attempted at above condition with FDH, AldDH, and ADH. A sample solution containing ZnTPPS ( $0.1 \mu\text{mol dm}^{-3}$ ),  $MV^{2+}$  ( $0.1 \text{ mmol dm}^{-3}$ ), TEOA ( $0.3 \text{ mol dm}^{-3}$ ) FDH (12.5 units), AldDH (12.5 units) and ADH (12.5 units) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 5 min.  $\text{NaHCO}_3$  ( $0.01 \text{ mmol dm}^{-3}$ ) was added to the sample solution and then irradiated. The produced methanol was measured by gas chromatography using a Sorbitol 25%-Gasport B column ( $2 \text{ m} \times 3 \text{ mm}$  i.d., GL Sciences) attached to a Shimadzu GC-8A gas chromatograph (oven temperature, 100 °C; carrier gas,  $\text{N}_2$ ; flow rate,  $21.8 \text{ mL min}^{-1}$ ). When the sample solution was irradiated with a 200-W tungsten lamp, methanol production is shown in



**Figure 1.** Time dependence of methanol production with the system consisting of TEOA, ZnTPPS,  $MV^{2+}$ ,  $\text{NaHCO}_3$ , FDH, AldDH, and ADH in potassium phosphate buffer (pH 8.0) under steady state irradiation at 30 °C.

Figure 1. The methanol production was  $0.55 \mu\text{mol dm}^{-3}$  after 4 h irradiation. The conversion yield of  $\text{HCO}_3^-$  to methanol was estimated to be 5.5% after 4-h irradiation. In contrast, no methanol production was observed without irradiation. Moreover, no methanol production was also observed in the absence of  $\text{HCO}_3^-$ . Thus, the produced methanol is not from the oxidized TEOA but the origin from  $\text{HCO}_3^-$  reduction with three dehydrogenases. These results indicate that the photochemical synthesis of methanol from  $\text{HCO}_3^-$  with FDH, AldDH and ADH via the photoreduction of  $MV^{2+}$  using ZnTPPS photosensitization. However, the amount of produced methanol and the turnover number of ZnTPPS and  $MV^{2+}$  are respectively low values. The improvement of the methanol production and optimum condition using the reaction system as shown in Scheme 1 is being studied in detail.

In conclusion, photochemical and enzymatic synthesis of methanol from  $\text{HCO}_3^-$  with dehydrogenases FDH, AldDH, and ADH and  $MV^{2+}$  photoreduction by the visible light photosensitization of ZnTPPS in the presence of TEOA was developed and the concentration of methanol from  $\text{HCO}_3^-$  was  $0.55 \mu\text{mol dm}^{-3}$  and the conversion yield of  $\text{HCO}_3^-$  to methanol was estimated to be 5.5% after 4-h irradiation. This system could be developed to convert  $\text{CO}_2$  into useful organic compound materials.

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