Photochemical and Enzymatic Synthesis of Methanol from HCO_3^- with Dehydrogenases and Zinc Porphyrin

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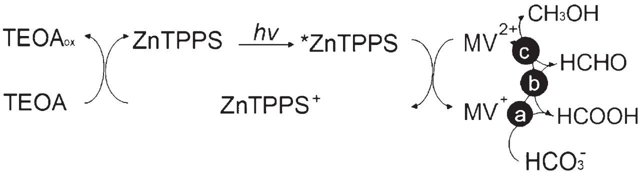
Photochemical and enzymatic methanol synthesis from HCO_3^- with formate dehydrogenase (FDH), aldehyde dehydrogenase (AldDH), and alcohol dehydrogenase (ADH) via the photoreduction of MV^{2+} using ZnTPPS photosensitization was developed and the amount of produced methanol was 0.55 μmol dm^{-3} after 4-h irradiation.

CO_2 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 led to investigate photocatalysis on semiconductors such as titanium dioxide, silicon 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), have got a 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 photosensitization of water-soluble zinc porphyrin and ferredoxin-$NADP^+$ reductase (FDR), and CO_2 reduction with FDH can be established.

Some studies relating 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 photosensitization of chemical-modified chlorophyll and FDR, and malic acid synthesis from pyruvate and HCO_3^- with malic enzyme has also been reported.[12] Enzymatic formic acid synthesis from HCO_3^- with FDH and MV^{2+} photoreduction with a system containing ruthenium(II) coordination compound as a photosensitiser and mercaptoethanol (RSH) as an electron donor also has been reported.[13] We previously reported the visible-light induced enzymatic formic acid synthesis from HCO_3^- with formate dehydrogenase (FDH) using the photosensitization of water-soluble zinc poirphyrin.[14–16] A photochemical methanol synthesis from 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 HCO_3^- as shown in Scheme 1 with three dehydrogenases mentioned above and photosensitizer such as zinc porphryin has not been developed yet.



Scheme 1. Photochemical and enzymatic methanol synthesis from HCO_3^- with FDH (a), AldDH (b), and ADH (c) via the photoreduction of MV^{2+} using ZnTPPS photosensitization.

In this letter, we describe a system for visible light-induced methanol synthesis from HCO_3^- with the system FDH, AldDH, and ADH, and 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 (H_2TPPS) 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 μmol formic acid to CO_2 per minute in the presence of NAD^+.

One unit of AldDH activity was defined as the amount of enzyme required to produce 1.0 μmol formaldehyde to formic acid per minute in the presence of NAD^+.

One unit of ADH activity was defined as the amount of enzyme required to produce 1.0 μmol methanol to formaldehyde per minute in the presence of NAD^+.

ZnTPPS was synthesized by refluxing H_2TPPS with excess molar of zinc acetate in methanol and then was evaporated to dryness in vacuo.[17]

Photoreduction of MV^{2+} is the most important step in photochemical synthesis of methanol from HCO_3^-system. To attain the highest yield of reduced MV^{2+} ($MV^{\bullet+}$), the reaction conditions of photoreduction of MV^{2+} consisting of TEOA and ZnTPPS were investigated. A solution containing ZnTPPS (0.1 μmol dm^{-3}), MV^{2+}, and TEOA (0.3 mol dm^{-3}) in 3.0 mL of 10 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 cm with a Toshiba L-39 cut-off filter at

30 °C. The $MV^{\bullet+}$ concentration was determined by the absorbance at 605 nm using the molar coefficient 13000 mol^{-1} dm^3 cm^{-1}.[18]

When the sample solution was irradiated, the accumulation of $MV^{\bullet+}$ was observed. The rate of $MV^{\bullet+}$ formation increased with the concentration of MV^{2+}. In contrast, the conversion yield of MV^{2+} to $MV^{\bullet+}$ increased with the concentration of MV^{2+} up to 0.1 mmol 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 $MV^{\bullet+}$ is important for formic acid synthesis using the system shown in Scheme 1. Therefore, the optimum MV^{2+} concentration is 0.1 mmol dm^{-3} for methanol synthesis. After 40 min irradiation, 25 µmol dm^{-3} $MV^{\bullet+}$ was produced. The reduction ratio of MV^{2+} to $MV^{\bullet+}$ 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 HCO_3^- was attempted at above condition with FDH, AldDH, and ADH. A sample solution containing ZnTPPS (0.1 µmol dm^{-3}), MV^{2+} (0.1 mmol dm^{-3}), TEOA (0.3 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. $NaHCO_3$ (0.01 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 m × 3 mm i.d., GL Sciences) attached to a Schimadzu GC-8A gas chromatograph (oven temperature, 100 °C; carrier gas, N_2; flow rate, 21.8 mL min^{-1}). When the sample solution was irradiated with a 200-W tungsten lamp, methanol production is shown in Figure 1. The methanol production was 0.55 µmol dm^{-3} after 4 h irradiation. The conversion yield of 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 HCO_3^-. Thus, the produced methanol is not from the oxidized TEOA but the origin from HCO_3^- reduction with three dehydrogenases. These results indicate that the photochemical synthesis of methanol from 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.

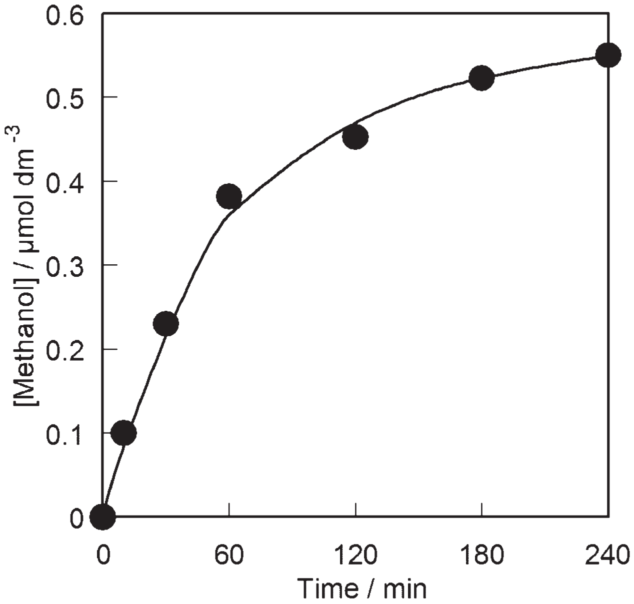


Figure 1. Time dependence of methanol production with the system consisting of TEOA, ZnTPPS, MV^{2+}, $NaHCO_3$, FDH, AldDH, and ADH in potassium phosphate buffer (pH 8.0) under steady state irradiation at 30 °C.

In conclusion, photochemical and enzymatic synthesis of methanol from 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 HCO_3^- was 0.55 µmol dm^{-3} and the conversion yield of HCO_3^- to methanol was estimated to be 5.5% after 4-h irradiation. This system could be developed to convert CO_2 into useful organic compound materials.

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