

## [Research Note]

## Photochemical Synthesis of Methanol from Formaldehyde Using Alcohol Dehydrogenase Coupled with Photosensitization of Zinc Porphyrin

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Photochemical synthesis of methanol from formaldehyde was evaluated with alcohol dehydrogenase (ADH) from *Saccharomyces cerevisiae* and  $\text{NAD}^+$  photoreduction by the visible light photosensitization of zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) in the presence of triethanolamine (TEOA) as an electron-donating reagent. Irradiation of a solution containing ZnTPPS, methylviologen ( $\text{MV}^{2+}$ ),  $\text{NAD}^+$ , diaphorase (5 units) and TEOA in potassium phosphate buffer with visible light resulted in formation of NADH increasing with time. NADH was not formed in the absence of any one of the five components, TEOA, ZnTPPS,  $\text{MV}^{2+}$ , diaphorase and  $\text{NAD}^+$ . The reduction ratio of  $\text{NAD}^+$  to NADH reached about 60% after 180 min irradiation. Irradiation of a solution containing formaldehyde, ZnTPPS,  $\text{MV}^{2+}$ , ADH, diaphorase and TEOA with visible light resulted in formation of methanol. The formaldehyde concentration decreased with formation of methanol. This result indicates that the photochemical synthesis of methanol from formaldehyde depends on ADH and NADH produced by the photosensitization of ZnTPPS. The concentration of methanol was  $0.38 \mu\text{mol} \cdot \text{dm}^{-3}$  after 3 h irradiation under conditions of ZnTPPS ( $1.0 \mu\text{mol} \cdot \text{dm}^{-3}$ ),  $\text{MV}^{2+}$  ( $0.1 \text{mmol} \cdot \text{dm}^{-3}$ ),  $\text{NAD}^+$  ( $0.1 \text{mmol} \cdot \text{dm}^{-3}$ ), diaphorase (5 units), TEOA ( $0.3 \text{mol} \cdot \text{dm}^{-3}$ ), formaldehyde ( $16 \mu\text{mol} \cdot \text{dm}^{-3}$ ) and ADH (25 units).

**Keywords**

Photochemical methanol synthesis, Zinc porphyrin, Alcohol dehydrogenase, Enzymatic synthesis

**1. Introduction**

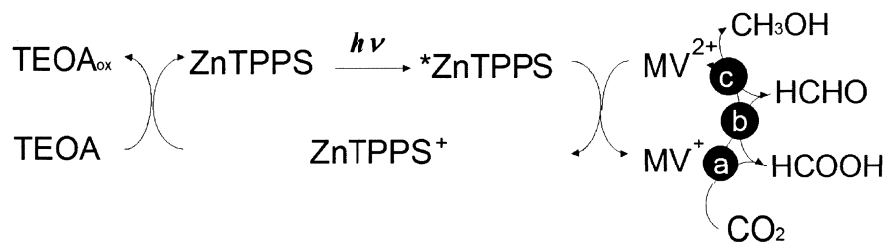
Biocatalytic methods have been widely investigated because of their regio- and stereo-selectivity and mild physiological conditions. Enzymatic methods could compete with conventional chemical methods, especially in organic syntheses that cannot be successfully carried out by chemical catalysts. Examples of beneficial biocatalytic reactions are oxidations of alkanes, alkenes, and aromatics<sup>1</sup>. As the biocatalytic reactions proceed in aqueous reaction medium, enzymatic methods are attractive for environment friendly processes in organic synthetic chemistry. Dehydrogenases such as lactate, formic, aldehyde and alcohol dehydrogenases are useful enzymes for the synthesis of valuable organic compounds such as lactic acid, methanol and so on. The coenzymes of these dehydrogenases are  $\text{NAD}^+$ , NADH and viologen derivatives. For example, alcohol dehydrogenase (ADH) catalyzes the reversible oxidation and reduction between alcohol and aldehyde with the redox of NADH or methylviologen ( $\text{MV}^{2+}$ ). Therefore, photochemical organic syntheses that couple a photoredox system such as photoreduction of  $\text{NAD}^+$  or  $\text{MV}^{2+}$  by the

sensitization of photosensitizer and a catalytic reaction with dehydrogenase can be developed.

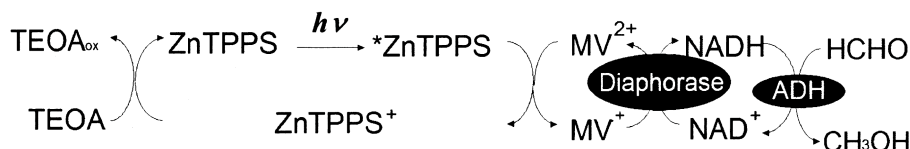
The visible light-induced photoredox systems consisting of an electron donor, a photosensitizer, and an electron relay, have been widely studied<sup>2</sup>. Photoredox systems with visible light include water-soluble zinc porphyrins with absorption bands in the visible light region (380–600 nm), which have been widely used as effective photosensitizers<sup>3</sup>. Zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP) are especially useful as photosensitizers<sup>4,5</sup>. We previously reported visible-light induced enzymatic formic acid synthesis from  $\text{HCO}_3^-$  with FDH using the photosensitization of water-soluble zinc porphyrin<sup>6,7</sup>. Malic acid synthesis combined with the photoreduction of  $\text{NADP}^+$  by the photosensitization of ruthenium(II) polypyridyl complex and ferredoxin- $\text{NADP}^+$  reductase (FNR), and malic acid synthesis from pyruvate and  $\text{HCO}_3^-$  with malic enzyme (ME) have also been reported<sup>8</sup>. We also reported the development of a photochemical and enzymatic methanol synthesis from  $\text{HCO}_3^-$  with formate dehydrogenase (FDH), aldehyde dehydrogenase (AldDH) and alcohol dehydrogenase (ADH) via the photoreduction of methylviologen ( $\text{MV}^{2+}$ ) using ZnTPPS photosensitization as shown in **Scheme 1**<sup>9</sup>. In this system, the conversion

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Scheme 1 Photochemical and Enzymatic Methanol Synthesis from  $\text{CO}_2$  with FDH (a), AldDH (b) and ADH (c) through the Photoreduction of  $\text{MV}^{2+}$  Using ZnTPPS Photosensitization



Scheme 2 Photochemical Methanol Synthesis from Formaldehyde with Alcohol Dehydrogenase (ADH) through the Photoreduction of  $\text{NAD}^+$  with Diaphorase Using ZnTPPS Photosensitization

yield of  $\text{HCO}_3^-$  to methanol is estimated to be 5.0% after 3 h irradiation. As reduced  $\text{MV}^{2+}$  ( $\text{MV}^{\bullet+}$ ) is used as a substrate of the dehydrogenase, the reduction ratio of  $\text{MV}^{2+}$  to  $\text{MV}^{\bullet+}$  is important for the most effective conversion yield of  $\text{HCO}_3^-$  to methanol. However, the reduction ratio of  $\text{MV}^{2+}$  to  $\text{MV}^{\bullet+}$  is only about 25% after 180 min irradiation. In contrast, diaphorase catalyzes the oxidation and reduction between  $\text{NADH}$  and  $\text{NAD}^+$  with the redox of an electron carrier molecule such as  $\text{MV}^{2+}$ . Therefore, more effective photochemical methanol synthesis could be achieved by addition of  $\text{NAD}^+$  and diaphorase in **Scheme 1**.

The present study tried to improve the yield of methanol production in **Scheme 1**, by investigating the effect of  $\text{NAD}^+$  and diaphorase addition for visible light-induced methanol synthesis from formaldehyde with ADH from *Saccharomyces cerevisiae* and visible light photosensitization of ZnTPPS in the presence of TEOA as an electron donor as shown in **Scheme 2**.

## 2. Experimental

### 2.1. Materials

Tetraphenylporphyrin-4-tetrasulfonate ( $\text{H}_2\text{TPPS}$ ) was obtained from Tokyo Chemical Industry Co., Ltd. ADH was obtained from Sigma Co., Ltd.  $\text{NAD}^+$  and diaphorase was purchased from Oriental Yeast Co., Ltd. The other chemicals were analytical grade or the highest grade available. One unit of ADH activity was defined as the amount of enzyme required to convert  $1.0 \mu\text{mol}$  methanol to formaldehyde per min in the presence of  $\text{NAD}^+$ .

### 2.2. Synthesis of ZnTPPS

ZnTPPS was synthesized by refluxing  $\text{H}_2\text{TPPS}$  with about 10 times molar equivalent of zinc acetate in

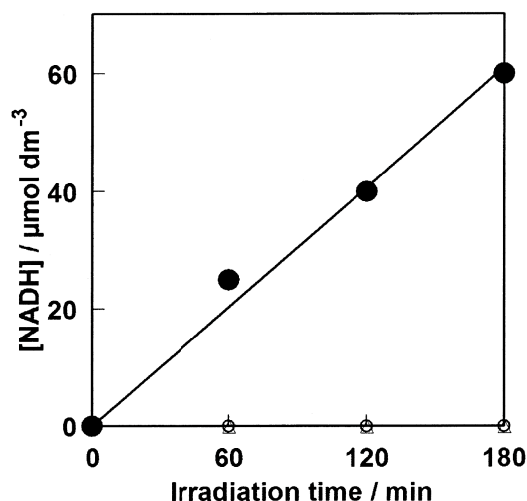
100 ml of methanol at  $60^\circ\text{C}$  for 2 h<sup>10</sup>. The insertion of zinc ion into  $\text{H}_2\text{TPPS}$  was monitored by UV-vis absorption spectroscopy using a Shimadzu Multispec 1500 spectrophotometer.

### 2.3. Photoreduction of $\text{NAD}^+$

The sample solution containing ZnTPPS ( $1.0 \mu\text{mol}\cdot\text{dm}^{-3}$ ),  $\text{MV}^{2+}$  ( $0.1 \text{mmol}\cdot\text{dm}^{-3}$ ),  $\text{NAD}^+$  ( $0.1 \text{mmol}\cdot\text{dm}^{-3}$ ), diaphorase (5 units) and TEOA ( $0.3 \text{mol}\cdot\text{dm}^{-3}$ ) in 3.0 ml of  $10 \text{mmol}\cdot\text{dm}^{-3}$  potassium phosphate buffer was deaerated by 6 freeze-pump-thaw cycles and then irradiated with a 200 W tungsten lamp (Philips) at a distance of 3.0 cm with a Toshiba L-39 cut-off filter at  $30^\circ\text{C}$ . To prevent ZnTPPS and ADH from degradation by near ultra-visible light, light wavelengths of less than 360 nm were removed by the Toshiba L-39 cut-off filter. The light intensity was measured by potassium ferrioxalate actinometry<sup>11</sup>. The reduction of  $\text{NAD}^+$  to  $\text{NADH}$  was determined by the specific absorption at 340 nm with a molar extinction coefficient of  $6.3 \times 10^3 \text{mol}\cdot\text{dm}^{-3}\cdot\text{cm}^{-1}$ .

### 2.4. Photochemical Methanol Synthesis from Formaldehyde

The sample solution containing ZnTPPS ( $1.0 \mu\text{mol}\cdot\text{dm}^{-3}$ ),  $\text{MV}^{2+}$  ( $0.1 \text{mmol}\cdot\text{dm}^{-3}$ ),  $\text{NAD}^+$  ( $0.1 \text{mmol}\cdot\text{dm}^{-3}$ ), diaphorase (5 units), TEOA ( $0.3 \text{mol}\cdot\text{dm}^{-3}$ ), and ADH (25 units) deaerated by 6 freeze-pump-thaw cycles and then flushed with argon gas for 5 min. Formaldehyde ( $16 \mu\text{mol}\cdot\text{dm}^{-3}$ ) was added to the sample solution and then irradiated. The methanol formed was measured by gas chromatography using a Sorbitol 25%-Gasport B column (2 m length, 3 mm i.d., GL Sciences) attached to a Shimadzu GC-8A gas chromatograph (oven temperature,  $100^\circ\text{C}$ ; carrier gas,  $\text{N}_2$ ; flow rate, 21.8 ml/min).



Open circle: absence of ZnTPPS. Open triangle: without irradiation.

Fig. 1 Time Dependence of NADH Production with the System Consisting of TEOA, ZnTPPS, MV<sup>2+</sup>, Diaphorase and NAD<sup>+</sup> under Steady State Irradiation (closed circle)

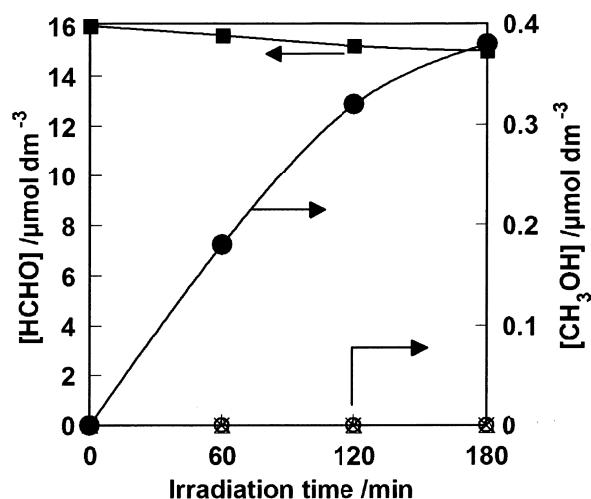
## 2. 5. By-product Analysis

By-product formation in the reaction mixture was analyzed using HPLC with an electrical conductivity detector (Shimadzu CDD-10A<sub>VP</sub>) (column temperature, 40°C; column, polystyrene sulfonate column Shimadzu SCR-H; elutant, *p*-toluene sulfonic acid; flow rate, 0.8 ml·min<sup>-1</sup>).

## 3. Results and Discussion

### 3. 1. Photoreduction of NAD<sup>+</sup>

Photoreduction of NAD<sup>+</sup> to NADH is the most important step in the photochemical synthesis of methanol. To attain the highest yield of NADH, the reaction conditions of photoreduction of NAD<sup>+</sup> to NADH consisting of TEOA and ZnTPPS were investigated. **Figure 1** shows the accumulation of NADH during irradiation of the sample solution containing ZnTPPS (1.0 μmol·dm<sup>-3</sup>), MV<sup>2+</sup> (0.1 mmol·dm<sup>-3</sup>), NAD<sup>+</sup> (0.1 mmol·dm<sup>-3</sup>), diaphorase (5 units) and TEOA (0.3 mol·dm<sup>-3</sup>) in 3.0 ml of 10 mmol·dm<sup>-3</sup> potassium phosphate buffer, as well as the time dependences of NADH accumulation in the absence of ZnTPPS (open circle) and without irradiation (closed triangle). In these cases, no NADH accumulation is observed. Moreover, NADH is not produced if even one of the five components, TEOA, ZnTPPS, MV<sup>2+</sup>, diaphorase and NAD<sup>+</sup>, was missing. After 180 min irradiation, 60 μmol·dm<sup>-3</sup> NADH was produced. The reduction ratio of NAD<sup>+</sup> to NADH was about 60%. The quantum yield of NAD<sup>+</sup> to NADH was 5.2% by potassium ferrioxalate actinometry after 180 min irradiation. In



Open circle: absence of ZnTPPS. Closed circle: without irradiation. x: absence of formaldehyde.

Fig. 2 Time Dependence of Methanol Formation (closed circle) and Formaldehyde Concentration (closed square) with the System Consisting of TEOA, ZnTPPS, MV<sup>2+</sup>, Diaphorase, NAD<sup>+</sup>, Formaldehyde and ADH under Steady State Irradiation

the absence of diaphorase and NAD<sup>+</sup>, the reduction ratio of MV<sup>2+</sup> to MV·<sup>+</sup> was about 25% after 180 min irradiation<sup>9</sup>). These results indicate that effective NAD<sup>+</sup> photoreduction is achieved using the photosensitization of ZnTPPS in the presence of diaphorase and this system is suitable for photochemical and enzymatic methanol synthesis.

### 3. 2. Photochemical Methanol Synthesis from Formaldehyde

Photochemical methanol synthesis from formaldehyde was attempted with ADH and the NAD<sup>+</sup> photoreduction system based on the photosensitization of ZnTPPS. **Figure 2** shows methanol production during irradiation of the sample solution containing ZnTPPS (1.0 μmol·dm<sup>-3</sup>), MV<sup>2+</sup> (0.1 mmol·dm<sup>-3</sup>), NAD<sup>+</sup> (0.1 mmol·dm<sup>-3</sup>), diaphorase (5 units), TEOA (0.3 mol·dm<sup>-3</sup>), formaldehyde (16 μmol·dm<sup>-3</sup>) and ADH (25 units) with a 200 W tungsten lamp. Methanol formation was 0.38 μmol·dm<sup>-3</sup> after 3 h irradiation. **Figure 2** also shows the time dependence of the formaldehyde concentration. The formaldehyde concentration decreased with increasing methanol formation. After 3 h irradiation, the formaldehyde concentration was 15.5 μmol·dm<sup>-3</sup> and 0.50 μmol·dm<sup>-3</sup> of formaldehyde was consumed. This result indicates that the photochemical synthesis of methanol from formaldehyde occurred through ADH and the NADH produced by the photosensitization of ZnTPPS.

**Figure 2** shows the time dependences of methanol accumulation in the absence of ZnTPPS, formaldehyde and without irradiation. In these cases, no methanol

accumulation was observed. Moreover, methanol was not formed if any one of the seven components, TEOA, ZnTPPS, MV<sup>2+</sup>, diaphorase, NAD<sup>+</sup>, formaldehyde and ADH, was missing. Therefore, no methanol was produced by the degradation of organic reagents such as TEOA and ADH. Gas chromatograph analysis detected methanol and formaldehyde but no other species. By-product formation in the reaction mixture was analyzed using HPLC with an electrical conductivity detector. NADH and ZnTPPS were detected, but not formic acid and hydrogen bicarbonate. Therefore, the methanol formed in the system containing the above seven components was attributed to formaldehyde reduction with ADH and NADH produced by the photosensitization of ZnTPPS in the presence of TEOA.

#### 4. Conclusion

Photochemical synthesis of methanol was achieved from formaldehyde with ADH and NAD<sup>+</sup> photoreduction formed by visible light photosensitization of ZnTPPS in the presence of TEOA. The concentration of methanol was 0.38  $\mu\text{mol} \cdot \text{dm}^{-3}$  after 3 h irradiation.

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#### 要 旨

#### 亜鉛ポルフィリンの光増感作用とアルコール脱水素酵素を利用したホルムアルデヒドからの光化学的メタノール合成

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常温常圧条件で、ホルムアルデヒドを還元してメタノールを合成する新規な反応系の確立を目的として、水溶性亜鉛ポルフィリンの可視光増感作用による NAD<sup>+</sup> の還元反応とアルコール脱水素酵素 (ADH) とを利用した、ホルムアルデヒドからの光学的メタノール合成反応系を構築した。トリエタノールアミン、亜鉛ポルフィリン、メチルビオローゲン、ホルムアルデ

ヒド、ジアホラーゼ、NAD<sup>+</sup> を含む系にタングステンランプを用い可視光照射したところ、NAD<sup>+</sup> が還元され NADH が生成した。光照射時間 3 時間後の NAD<sup>+</sup> から NADH への変換効率は約 60% であった。この系に ADH を添加し、光照射すると定量的にメタノールが生成した。光照射 3 時間後のメタノール生成量は 0.38  $\mu\text{mol} \cdot \text{dm}^{-3}$  であった。