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Photochemical and enzymatic synthesis of methanol from formaldehyde with alcohol dehydrogenase from *Saccharomyces cerevisiae* and water-soluble zinc porphyrin

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Abstract

We evaluated the photochemical and enzymatic synthesis of methanol from formaldehyde with alcohol dehydrogenase (ADH) from *Saccharomyces cerevisiae* and NAD^+ photoreduction by the visible-light sensitization of zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) in the presence of methylviologen (MV^{2+}), diaphorase, and triethanolamine (TEOA). When the sample solution containing ZnTPPS, MV^{2+}, NAD^+, diaphorase, and TEOA in potassium phosphate buffer solution was irradiated, the NADH produced increased with the irradiation time. After irradiation for 180 min, the conversion yield of NAD^+ to NADH was about 60% under 0.1 mM NAD^+ condition. The methanol production also depended on the conversion yield of NAD^+ to NADH. After irradiation for 180 min, 0.38 μM of methanol was produced from formaldehyde (16 μM). The conversion ratio of formaldehyde to methanol was about 2.3%. This result indicates that a system for the photochemical synthesis of methanol from formaldehyde was developed with ADH and the NADH produced by the photosensitization of ZnTPPS in water media.

Keywords: Photochemical methanol synthesis; Zinc porphyrin; Alcohol dehydrogenase; Enzymatic synthesis; Water media

1. Introduction

Organic syntheses based on biocatalytic methods with regio- and stereo-selectivity in mild physiological conditions have been widely studied. Enzymatic synthesis competes with conventional chemical synthesis, especially in organic syntheses that cannot be successfully carried out by chemical catalysts. The oxidation of alkanes, alkenes, and aromatic molecules proceeds easily by the biocatalytic method [1]. Since biocatalytic reactions proceed in aqueous reaction media, enzymatic methods are attractive as green processes for organic chemical synthesis. Dehydrogenases such as lactate, formate, aldehyde, and alcohol dehydrogenases are useful enzymes for the synthesis of valuable organic compounds such as lactic acid, formic acid, and methanol. The coenzymes of these dehydrogenases are NAD^+, NADH, and viologen derivatives. Thus, the photochemical and enzymatic organic synthesis system can be developed by the combination of these dehydrogenases and the redox reaction of the coenzymes with the visible-light sensitization of photosensitizers. Visible-light-induced photoredox systems comprising an electron donor, photosensitizer, and electron relay have been widely studied [2–4]. Photoredox systems with visible light, such as water-soluble zinc porphyrins, have absorption bands in the visible-light region (380–600 nm); these porphyrins have been widely used as effective photosensitizers [5–7]. Zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP) are especially useful as photosensitizers [8–10]. Some studies on the photochemical and enzymatic synthesis of organic molecules have been reported [11–18]. Previously, we reported the visible-light-induced enzymatic formic acid synthesis from HCO_3^- with formate dehydrogenase (FDH) using the photosensitization of water-soluble zinc porphyrin [11–13]. We also reported the development of photochemical and enzymatic methanol synthesis from HCO_3^- with FDH, aldehyde dehydrogenase (AldDH), and alcohol dehydrogenase (ADH) via the photoreduction of methylviologen (MV^{2+}) using ZnTPPS photosensitization [17]. For example, ADH catalyzes the oxidation and

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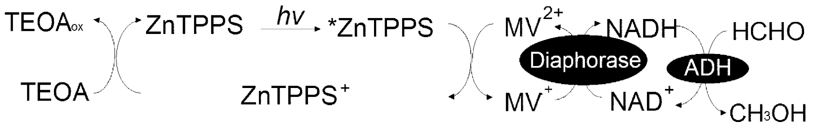

Scheme 1. Photochemical methanol synthesis from formaldehyde with alcohol dehydrogenase (ADH) via the photoreduction of NAD^+ with diaphorase using ZnTPPS photosensitization.

reduction between alcohol and aldehyde by the redox reaction of NADH or MV^{2+}. Formaldehyde, a typical pollutant that originates from furnishing and decorating materials, frequently causes cancer. In the gas phase, it can be easily decomposed by UV irradiation in the presence of a photocatalyst such as $Pt/Fe_2O_3/TiO_2$ [19]. Formaldehyde exists stably in aqueous media as formaline, which is still widely used for preserving biological samples. Thus, the visible-light-induced methanol synthesis from formaldehyde in water media using the coupling of the photoredox system and ADH will be attractive for the utilization of waste water. The reduction ratio of MV^{2+} to MV^+ is an important factor in photochemical and enzymatic organic synthesis that use the reduced MV^{2+} (MV^+) as a substrate for these NAD^+-dependent dehydrogenases. The diaphorase catalyzes the oxidation and reduction between NADH and NAD^+ with the redox reaction of MV^{2+}. Therefore, the photochemical methanol synthesis from formaldehyde that couples the photoredox system – such as the reduction of NAD^+ with photsensitizer, MV^{2+}, diaphorase, and NAD^+ – and the enzymatic process with ADH will be achieved as shown in Scheme 1.

In this study, we describe a system for visible-light-induced methanol synthesis from formaldehyde with ADH from *Saccharomyces cerevisiae* and NAD^+ photoreduction by the visible-light photosensitization of ZnTPPS in the presence of TEOA as an electron donor, as shown in Scheme 1.

2. Experimental

2.1. Materials

Tetraphenylporphyrin-4-tetrasulfonate (H_2TPPS) was obtained from Tokyo Kasei Co. Ltd. ADH was obtained from Sigma Co. Ltd. NAD^+ and diaphorase were obtained from Oriental Yeast Co. Ltd. The other chemicals were of analytical grade or the highest grade available. One unit of ADH activity was defined as the amount of enzyme required to convert 1.0 μmol methanol to formaldehyde per minute in the presence of NAD^+.

2.2. Synthesis of ZnTPPS

According to the previously reported method, ZnTPPS was synthesized by refluxing H_2TPPS with about 10 times molar equivalent of zinc acetate in 100 ml of methanol at 60 °C for 2 h [17]. The introduction of zinc ion into H_2TPPS was monitored by UV–vis absorption spectroscopy using a Shimadzu Multispec 1500 spectrophotometer.

2.3. Photoreduction of NAD^+

The sample solution contained ZnTPPS (1.0 μM), MV^{2+} (0.1 mM), NAD^+, diaphorase (five units), and TEOA (0.3 M) in 3.0 ml of 10 mM potassium phosphate buffer (pH 8.0). It was deaerated by freeze-pump-thaw cycles and then irradiated with a 200 W tungsten lamp (Philips) placed at a distance of 3.0 cm with a Toshiba L-39 cut-off filter at 30 °C. To prevent the degradation of ZnTPPS and ADH by near ultra-visible light, light wavelengths of less than 360 nm were eliminated by the Toshiba L-39 cut-off filter. The light intensity was measured by the potassium ferrioxalate actinometry method [18]. The reduction of NAD^+ to NADH was determined by the specific absorption at 340 nm with a molar extinction coefficient of $6.3 \times 10^3\ \mathrm{M\,cm^{-1}}$. To investigate the effect of NAD^+ concentration on the NAD^+ photoreduction rate, the concentration of NAD^+ was changed between 0.1 and 10 mM.

2.4. Photochemical and enzymatic methanol synthesis from formaldehyde

The sample solution containing ZnTPPS (1.0 μM), MV^{2+} (0.1 mM), NAD^+, diaphorase (five units), TEOA (0.3 M), and ADH (25 units) in 3.0 ml of 10 mM potassium phosphate buffer (pH 8.0) was deaerated by freeze-pump-thaw cycles and then flushed with argon gas for 5 min. Formaldehyde (16 μM) was added to the sample solution and then irradiated. The methanol produced was measured by gas chromatography using a Sorbitol 25%-Gasport B column (2 m × 3 mm i.d., GL Sciences) attached to a Shimadzu GC-8A gas chromatograph (oven temperature: 100 °C; carrier gas: N_2; flow rate: $21.8\ \mathrm{ml\,min^{-1}}$). To investigate the effect of NAD^+ concentration on the methanol synthesis, the concentration of NAD^+ was changed between 0 and 10 mM.

2.5. By-product analysis

By-product formation in the reaction mixture was analyzed using an 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: $0.8\ \mathrm{ml\,min^{-1}}$).

3. Results and discussion

3.1. Photoreduction of NAD^+

The photoreduction of NAD^+ to NADH is the most important process in the photochemical synthesis of methanol. The

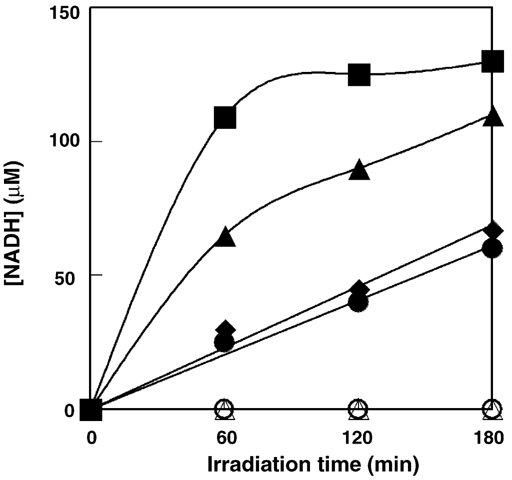

Fig. 1. Time dependence of NADH production with the system consisting of TEOA, ZnTPPS, MV^{2+}, diaphorase and NAD^+ under steady state irradiation. $[NAD^+] = 0.1$ mM (closed circle), 1.0 mM (closed diamond), 5.0 mM (closed triangle) and 10 mM (closed square). Open circle: in the absence of ZnTPPS. Open triangle: without irradiation.

reaction conditions necessary to obtain the highest NADH yield were investigated. Fig. 1 shows the formation of NADH during the irradiation of the sample solution consisting of ZnTPPS (1.0 μM), MV^{2+} (0.1 mM), NAD^+, diaphorase (five units), and TEOA (0.3 M) in 3.0 ml of 10 mM potassium phosphate buffer (pH 8.0). The rate of NADH formation increased with the concentration of NAD^+. In contrast, the conversion yield of NAD^+ to NADH decreased with the concentration of NAD^+, as shown in Fig. 2. ADH catalyzes both the reduction of formaldehyde to methanol and the oxidation of methanol to formaldehyde. In general, the reaction rate for the reduction of formaldehyde to methanol is slower than that for the oxidation of methanol to formaldehyde in the system with ADH. At high NAD^+ concentrations, formaldehyde is produced by the oxidation of methanol with ADH. The conversion yield of NAD^+ to NADH is an important factor for methanol synthesis using the system shown in Scheme 1. The optimum NAD^+ concentration was found to be 0.1 mM for methanol synthesis with ADH. After irradiation for 180 min, 60 μM NADH was produced and the reduction ratio of NAD^+ to NADH was about 60% under 0.1 mM NAD^+ condition. Potassium ferrioxalate actinometry revealed that the quantum yield of NAD^+ photoreduction was 5.2% after irradiation for 180 min. In the absence of diaphorase and NAD^+, the reduction ratio of MV^{2+} to MV^+ was ca. 25% after irradiation for 40 min [17]. These results indicate that an effective NAD^+ photoreduction system was developed using the photosensitization of ZnTPPS in the presence of diaphorase. The time dependences of NADH accumulation in the absence of ZnTPPS (open circle) and without irradiation (closed triangle) are also shown in Fig. 1. In these cases, no NADH accumulation was observed. Moreover, NADH was not produced if even one of the five components – TEOA, ZnTPPS, MV^{2+}, diaphorase, and NAD^+ – was missing.

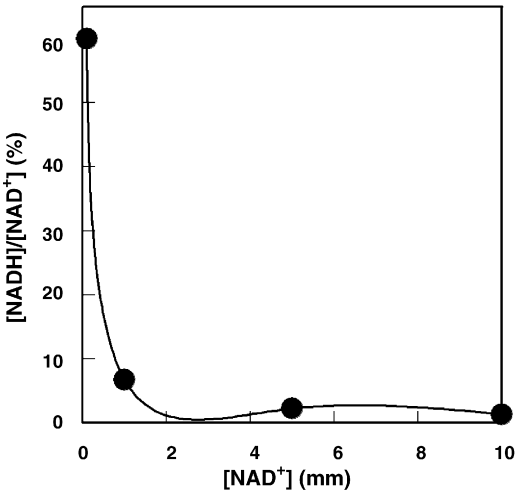

Fig. 2. Relationship between the conversion ratio of NAD^+ to NADH and the concentration of NAD^+.

3.2. Photochemical and enzymatic methanol synthesis from formaldehyde

Since we were able to accomplish an NAD^+ photoreduction system with the photosensitization of ZnTPPS, the photochemical and enzymatic methanol synthesis from formaldehyde was attempted with ADH.

Initially, the optimum ADH activity was determined under the condition of NADH (1.0 mM), formaldehyde (16 μM), and ADH in potassium phosphate buffer (pH 8.0) at 30 °C for 30 min incubation. The ADH activity was changed between 1.0 and 50 units. Fig. 3 shows the relationship among the ADH activity, produced methanol concentration (square), and reaction yield with regard to ADH activity (circle) after 30 min incubation. It was observed that the optimum methanol production (1.0 μM) occurred at 25 units of ADH. Methanol production increased with ADH activity up to 25 units and then decreased, as shown in Fig. 4 (square). In contrast, the reaction

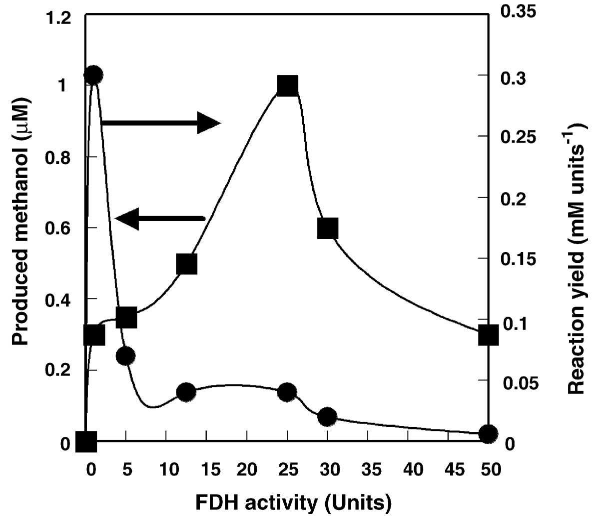

Fig. 3. Relationship among the ADH activity, produced methanol concentrations (square) and reaction yields toward ADH activity (circle) after 30 min incubation. The reaction condition was NADH (1.0 mM), formaldehyde (16 μM) and ADH in potassium phosphate buffer (pH 8.0) at 30 °C in dark.

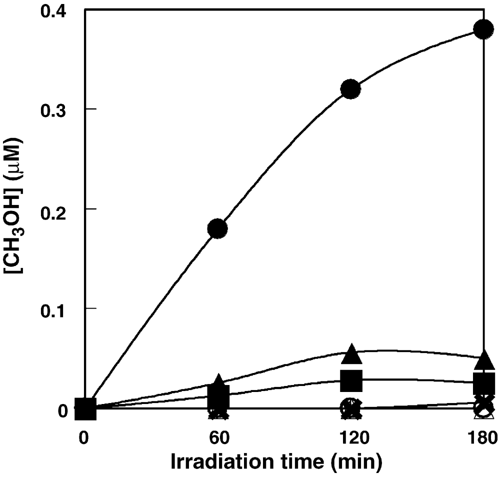

Fig. 4. Time dependence of methanol (closed circle) and formaldehyde concentration (closed square) with the system consisting of TEOA, ZnTPPS, MV^{2+}, diaphorase, NAD^+, formaldehyde and ADH under steady state irradiation. $[NAD^+] = 0.1$ mM (closed circle), 5.0 mM (closed triangle) and 10 mM (closed square). Open circle: in the absence of ZnTPPS. Open triangle: without irradiation. ×: in the absence of diaphorase and NAD^+.

yield decreased with an increase in ADH activity (circle in Fig. 4). However, the concentration of methanol produced was an important factor in the system, as shown in Scheme 1. Therefore, it was determined that the optimum ADH activity was 25 units.

Fig. 4 shows the methanol production during irradiation of the sample solution consisting of ZnTPPS (1.0 μM), MV^{2+} (0.1 mM), NAD^+, diaphorase (five units), TEOA (0.3 M), formaldehyde (16 μM), and ADH (25 units) with a 200 W tungsten lamp. The concentration of NAD^+ was changed between 0.1 and 10 mM. The rate of methanol production decreased with the concentration of NAD^+. The methanol production depended on the conversion yield of NAD^+ to NADH. At 0.1 mM of NAD^+, methanol production was 0.38 μM after irradiation for 180 min. The turnover numbers of ZnTPPS, MV^{2+}, and NAD^+ in the system containing ZnTPPS (1.0 μM), MV^{2+} (0.1 mM), NAD^+(0.1 mM), diaphorase (five units), TEOA (0.3 M), formaldehyde (16 μM), and ADH (25 units) were 0.13, 0.0013, and 0.0013 h^{-1}, respectively. Since the total turnover numbers for all the cofactors remained low, the photochemical synthesis system of methanol from formaldehyde was developed with ADH and the NADH produced by the photosensitization of ZnTPPS. The time dependences of the methanol accumulation in the absence of ZnTPPS (open circle) and without irradiation (open triangle) are also shown in Fig. 4. In these cases, no methanol accumulation was observed. Moreover, methanol was not produced if any one of the seven components – TEOA, ZnTPPS, MV^{2+}, diaphorase, NAD^+, formaldehyde, and ADH – was missing. Methanol production was observed in the absence of diaphorase and NAD^+, as shown in Fig. 4 (×). The reduced form of MV^{2+} is the substrate for NAD^+-dependent enzymes such as ADH and FDH. However, the amount of methanol production was small in comparison to that for the system in the presence of diaphorase and NAD^+. NADH is a natural substrate for ADH; therefore, in comparison to MV^+, NADH is a more suitable cofactor for methanol production from formaldehyde with ADH. Diaphorase catalyzes the transformation of NAD^+ to NADH, as shown in Scheme 1. Thus, methanol production from formaldehyde with ADH effectively proceeded by the addition of an NAD^+ reduction system with diaphorase. These results indicate that an effective methanol production system could be developed via NAD^+ photoreduction with the photosensitization of ZnTPPS in the presence of diaphorase. By-product formation in the reaction mixture was analyzed using a gas chromatograph and HPLC with an electrical conductivity detector. Methanol and formaldehyde were detected by gas chromatograph analysis, and NADH, NAD^+, and ZnTPPS were detected by HPLC analysis; however, formic acid and hydrogen bicarbonate were not detected.

4. Conclusion

The photochemical and enzymatic synthesis of methanol from formaldehyde with ADH and NAD^+ photoreduction by the visible-light photosensitization of ZnTPPS in the presence of MV^{2+}, diaphorase, and TEOA was developed. The rate of NADH formation increased with the concentration of NAD^+. However, the conversion yield of NAD^+ to NADH decreased with the concentration of NAD^+. The conversion yield of NAD^+ to NADH was the most important factor for methanol synthesis in Scheme 1. After irradiation for 180 min, the reduction ratio of NAD^+ to NADH was about 60% under 0.1 mM NAD^+ condition. The optimum NAD^+ concentration was 0.1 mM for methanol synthesis with ADH and 0.38 μM of methanol was produced after irradiation for 180 min. The conversion ratio of formaldehyde to methanol was 2.3% (initial concentration of formaldehyde was 16 μM). This result indicates that a photochemical synthesis system of methanol from formaldehyde can be developed with ADH and the NADH produced by the photosensitization of ZnTPPS.

Acknowledgement

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