



# Ethanol synthesis based on the photoredox system consisting of photosensitizer and dehydrogenases

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## ABSTRACT

Photochemical synthesis of ethanol from acetate was investigated with the system aldehyde (AldDH) and alcohol (ADH) dehydrogenases from Yeast, and methylviologen ( $MV^{2+}$ ) photoreduction by the visible light photosensitization of chlorophyll derivative, chlorin- $e_6$  of zinc complex (ZnChl- $e_6$ ) in the presence of NADPH as an electron donating reagent. Irradiation of a solution containing NADPH, ZnChl- $e_6$ ,  $MV^{2+}$ , AldDH, ADH and sodium acetate with visible light resulted in ethanol synthesis. The concentration of ethanol produced was 1.4 mM after 150 min irradiation under the condition of NADPH (3.3 mM), ZnChl- $e_6$  (100  $\mu$ M),  $MV^{2+}$  (12 mM), AldDH (0.22  $\mu$ M), ADH (6.7 nM) and sodium acetate (30 mM) in 50 mM of sodium pyrophosphate buffer pH 7.4.

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## 1. Introduction

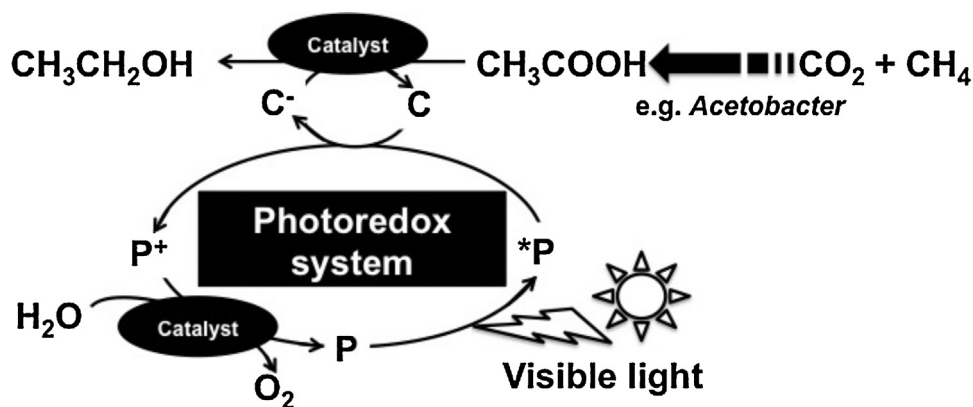
Photoredox system consisting of an electron donor, a photosensitizer, an electron carrier and catalyst is widely used for the photochemical conversion system [1–5]. This system is a simplification of the photosynthesis reaction, thus, is used as a model of artificial photosynthesis. By using reduced form of electron carrier molecule as a substrate for the catalyst in this system, hydrogen production and carbon dioxide conversion systems would be developed. For photoinduced hydrogen production systems, platinum nano-particle and enzyme, hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) have been widely used as catalysts [6–10]. The photochemical and enzymatic synthesis of organic compounds has also been developed using this system in the presence of various enzymes [11–22]. Dehydrogenase enzymes such as lactate (LDH), formate (FDH), aldehyde (AldDH) and alcohol dehydrogenase (ADH) are useful enzymes for the synthesis of valuable organic compounds such as lactic acid, formic acid, methanol etc. For example, we reported the lactic acid production with L-lactate

dehydrogenase from Pig heart (LDH) and reduced methyl viologen ( $MV^{2+}$ ) produced by the visible light photosensitization of water soluble zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP) and accomplishment of 17.0% yield of pyruvic acid to lactic acid, after 4 h irradiation [22]. The advantage of the combination of photoredox system and enzyme is that only the desired product will be obtained with the visible light energy.

In the photoredox system, the effective photosensitizer also is an essential component. Ruthenium(II) coordination compounds and porphyrins are widely used as effective photosensitizers [23,24]. As water soluble zinc porphyrins, zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and zinc tetrakis(4-methylpyridyl) porphyrin (ZnTMPyP), have 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 [25–28]. In contrast, photosynthesis dyes such as chlorophylls and its derivatives play important roles in light-harvesting, photoinduced energy transfer and charge separation in photosynthesis reactions. These dyes have the absorption maxima at 670–800 nm and the molar absorption coefficient of these dyes is larger than that of zinc porphyrin or ruthenium(II) coordination compound [29–31]. However, typical chlorophyll, Mg chlorophyll-*a* (Mg Chl-*a*) purified from green plant is unstable against the irradiation. On the other hand, zinc bacteriochlorophyll *a* (Zn BChl-*a*) was founded in an aerobic bacterium *Acidiphilium rubrum* [32]. As

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**Fig. 1.** Concept of visible-light induced ethanol synthesis from carbon dioxide, water and methane with the coupling system of photoredox, catalysts and biological materials.

the zinc porphyrins are stable against the irradiation and effective photosensitizers, zinc chlorophylls are attractive compound as a stable visible photosensitizer. Some studies on the preparation and characterization of the zinc chlorophyll and bacteriochlorophylls have been reported [33,34]. As chlorophylls and bacteriochlorophylls are insoluble to aqueous solution, chlorin- $e_6$  (Chl- $e_6$ ) formed by the hydrolysis of chlorophyll-a has three carboxylate groups in molecule. Zinc complex of Chl- $e_6$  (Zn Chl- $e_6$ ) also is attractive compound as an effective visible light photosensitizer with higher photostability for photoredox system.

Recently, ethanol is paid much attention to the lower carbon fuel. The largest single use of ethanol is as a motor fuel and fuel additive. Thus, we devoted to the synthesis of ethanol from carbon dioxide, natural gas such as methane and water with coupling the photoredox system, some catalysts and biological material as shown in Fig. 1. One of the important steps is the photochemical ethanol synthesis from acetate using combination of photoredox and catalyst system. By using natural co-enzyme nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) or its' reduced form ( $\text{NADH}$ ), AldDH catalyses both the reduction of acetate to acetaldehyde and the oxidation of acetaldehyde to acetate, and ADH also catalyses both the reduction of acetaldehyde to ethanol and the oxidation of ethanol to acetaldehyde as shown in Fig. 2. Thus, it is necessary to suppress the oxidation of acetaldehyde to acetate with AldDH and the oxidation of ethanol to acetaldehyde with ADH for the conversion of acetate to ethanol.

On the other hand, we found that the reduced form of  $\text{MV}^{2+}$  only acts as an artificial coenzyme for ADH and AldDH, and  $\text{MV}^{2+}$  is not recognized for artificial coenzyme of ADH and AldDH. Thus, the oxidation of acetaldehyde to acetate with AldDH and the oxidations of ethanol to acetaldehyde with ADH suppressed using  $\text{MV}^{2+}$  as an electron carrier as shown in Fig. 2. As  $\text{MV}^{2+}$  easily is reduced using photoredox system, moreover,  $\text{MV}^{2+}$  is suitable molecule for the photochemical ethanol synthesis from acetate with AldDH and ADH.

For water photolysis system, development of the optimal catalyst for oxidizing water especially attracts expectation. Among some catalysts for water photolysis, Chloroplast from green plant is attracting attention as an oxidation catalyst of water. Chloroplast has two important sites. One is the photosystem I with reaction center P700, acts as a photoreduction of  $\text{NADP}^+$  to  $\text{NADPH}$ . The other one is the photosystem II with reaction center P680, acts as an oxygen production based on the water oxidation. By using the  $\text{NADP}^+/\text{NADPH}$  redox coupling, the combination system of water photolysis using Chloroplast (process 1) and the photochemical ethanol synthesis from acetic acid with ZnChl- $e_6$ ,  $\text{MV}^{2+}$ , AldDH, and ADH (process 2) will be developed as shown in Fig. 3.

Here we describe a development of the photochemical ethanol synthesis from acetic acid with AldDH and ADH, and reduced  $\text{MV}^{2+}$

produced by the visible light photosensitization of ZnChl- $e_6$  in the presence of  $\text{NADPH}$  as an electron donor in the process 2 of Fig. 3. The principle of this photoredox system is as follows. When the visible-light is irradiated to Zn Chl- $e_6$ , Zn Chl- $e_6$  in photoexcited state ( $^*\text{Zn Chl-}e_6$ ) is formed. In the next step, reduced  $\text{MV}^{2+}$  ( $\text{MV}^{\bullet+}$ ) is produced by the electron transfer from  $^*\text{Zn Chl-}e_6$  to  $\text{MV}^{2+}$ . Subsequently,  $\text{MV}^{\bullet+}$  acts on AldDH and ADH as a co-enzyme, acetate is converted to ethanol via acetaldehyde. Finally, the electron transfers to the one-electron oxidized form of Zn Chl- $e_6$  (Zn Chl- $e_6^+$ ) from  $\text{NADPH}$  as an electron donor, Zn Chl- $e_6$  in the ground state is produced. In this paper, the investigation for the optimum condition of methanol synthesis from acetic acid using the system as shown in Fig. 2 also was studied.

## 2. Experimental

### 2.1. Materials

Chl- $e_6$  and sodium acetate, sodium pyrophosphate are purchased from Wako Pure Chemical Industries Ltd. Methylviologen dichloride ( $\text{MV}^{2+}$ ) is supplied by Tokyo Kasei Co. Ltd.  $\text{NADH}$  and  $\text{NADPH}$  are purchased from Oriental Yeast Co. Ltd. AldDH from Yeast (molecular weight 200 kDa) is obtained from Roche Co. Ltd. ADH from Yeast (molecular weight 80 kDa) is purchased from Oriental Yeast Co. Ltd. The other chemicals are analytical grade or the highest grade available.

### 2.2. Preparation of Zn Chl- $e_6$

Zn Chl- $e_6$  was synthesized by refluxing Chl- $e_6$  with about 10 times the molar equivalent of zinc acetate in 100 ml methanol at  $60^\circ\text{C}$  for 2 h according to the previously reported method [35–38]. The production of Zn Chl- $e_6$  was monitored by UV–vis absorption spectrum using Shimadzu Multispec 1500 spectrophotometer. The characteristic absorption bands of Zn Chl- $e_6$  at 418 and 638 nm increased and the absorbance at 400, 514 and 660 nm due to Chl- $e_6$  decreased gradually during reaction. After the mixture was cooled to room temperature and then the solvent was removed under vacuum. To remove the unreacted zinc acetate hydrate, the reaction mixture was washed with water and Zn Chl- $e_6$  was precipitated in water. Zn Chl- $e_6$  was collected by filtration and washed with water and then the purification was performed by recrystallization from water-methanol (5:1) solution.

### 2.3. Photoreduction of $\text{MV}^{2+}$

A solution containing Zn Chl- $e_6$  (100  $\mu\text{M}$ ),  $\text{MV}^{2+}$ , and  $\text{NADPH}$  (3.3 mM) in 3.0 ml of 50 mM sodium pyrophosphate buffer (pH 7.4) was deaerated by freeze–pump–thaw cycles repeated 6 times. The

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