

Visible Light-induced H₂ Production from the Bioresources Using Photosensitization of Zn Chlorophyll-*a*

Yumi Takeuchi* and Yutaka Amao

Department of Applied Chemistry, Oita University,
DannoHaru 700, Oita 870-1192, Japan
Fax: 81-97-554-7972, e-mail: takeuchi@cc.oita-u.ac.jp

The photoinduced H₂ production from the sucrose using photosensitization of artificial Zn chlorophyll-*a* by visible light was developed. Photostability of Zn chlorophyll-*a* was investigated by the absorbance changes. After 60 min irradiation, the absorbance decrease of Zn and Mg chlorophyll-*a* were 26 and 40 %, respectively. Zn chlorophyll-*a* has highly photostability compared with that of Mg chlorophyll-*a*. In H₂ production, a solution containing of sucrose, invertase, NAD⁺, glucose dehydrogenase (GDH), chlorophyll-*a* (Zn or Mg chlorophyll-*a*), methyl viologen (MV²⁺) and colloidal Pt was irradiated by visible light, and amounts of H₂ production using Zn chlorophyll-*a* was estimated to be 7.2 μmol after 240 min irradiation.

Key words: H₂ production, biomass, Zn chlorophyll-*a*, photoreduction

1. INTRODUCTION

Recently, for the problem of fossil resources exhausted and global warming, the utilization of the biomass contained in drainage, wood waste, etc, has been much paid attention as renewal energy source. Thus, the H₂, which is expected as clean energy, storage system will be developed by the combination of photoinduced water decomposition and biomass resources such as sucrose [1-5]. Some studies on H₂ production from glucose using an enzymatic pathway have been reported [6-10]. H₂ production from glucose using a combination of glucose dehydrogenase (GDH) and hydrogenase has been reported [11,12].

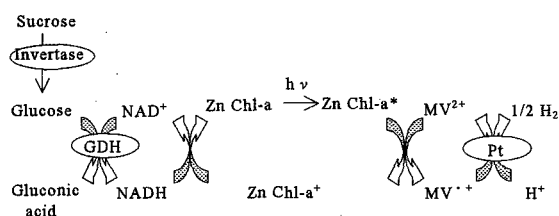
The photoinduced H₂ production consisting of electron donor, photosensitizer, electron carrier, and catalyst is widely used [13-19]. Porphyrins are widely used for the photosensitizer. However, molar absorption coefficient of porphyrins in visible light region was lower, thus porphyrins was ineffective photosensitization. Chlorophyll-*a* was effective photosensitization because the molar absorption coefficient in visible region was higher than that of porphyrin. Zn chlorophyll-*a* was a model of Zn bacteriochlorophyll-*a*, that was discovered in Acidophilic Bacterium *A. rubrum* [4,20,21], and is expected that superior to Mg chlorophyll-*a* in photostability.

In this work, the photoinduced H₂ production from sucrose using the reduction of NAD⁺ by glucose dehydrogenase, photoreduction of methylviologen (MV²⁺) using photosensitization of artificial Zn chlorophyll-*a* and H₂ production by colloidal Pt was developed.

2. MATERIALS AND EXPERIMENTS

Mg chlorophyll-*a* from *spirulina*, invertase from yeast, and GDH from *Bacillus* sp. were purchased from

Wako pure Chemical Industries Ltd. NAD⁺ and NADH were purchased from Oriental Yeast Co. Ltd. MV²⁺ and cetyltrimethylammonium bromide (CTAB) were purchased from Tokyo Kasei Co. Ltd. Hydrogen hexachloroplatinate hexahydrate and sodium citrate dihydrate were obtained from Kanto Chemical Co. Ltd. The other chemicals were analytical grade or the highest grade available. One unit of GDH activity was defined as the amount of enzyme that reduced 1.0 μmol of NAD⁺ to NADH by glucose per minute. One unit of invertase activity was defined as the amount of enzyme that produced 1.0 μmol of glucose by sucrose per minute.



Scheme 1. Visible light-induced H₂ production from sucrose using the system containing Zn chlorophyll-*a* (Zn Chl-*a*), invertase, glucose dehydrogenase (GDH), NAD⁺, methyl viologen (MV²⁺) and colloidal Pt.

Zn chlorophyll-*a* was synthesized by refluxing Mg chlorophyll-*a* with about 10 mol equiv of zinc acetate in 100 mL of methanol at 80 °C for 5 h. The insertion of zinc ion into chlorophyll-*a* ring was monitored by UV-vis absorption spectroscopy. During the reaction, chlorophyll-*a* of absorption maximum changed. After the reaction, methanol was removed by rotary evaporation and the product was with water. Then, the solvent was performed by recrystallization. Zn and Mg

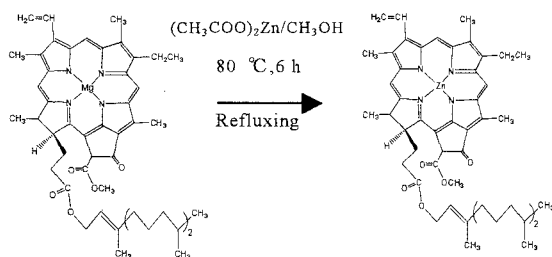


Fig. 1 Preparation of Zn chlorophyll-*a* from Mg chlorophyll-*a*

chlorophyll-*a* was solubilized with 10 mmol dm^{-3} CTAB.

Colloidal Pt was prepared with reduction of hexachloroplatinate solution with sodium citrate [18]. Water, 400 mL, containing 30 mg hydrogen hexahydrate was stirred for 1.5 h, and then a solution of 30 mL of water containing 600 mg of sodium citrate dehydrate was added and refluxed with a magnetic stirrer at $100\text{ }^\circ\text{C}$ for 4 h. The particle size of colloidal Pt prepared was estimated to be 1.5 nm. In general, the colloidal Pt activity decreased with increase the particle size (more than 2 nm). The prepared colloidal Pt has the ability to release $0.7\text{ }\mu\text{mol H}_2$ in the reaction system of $10\text{ }\mu\text{l}$ colloidal Pt, $1.2 \times 10^{-5}\text{ mmol}$ of sodium dithionite in 4 ml 50 mM Tris/HCl buffer (pH 7.4) at $30\text{ }^\circ\text{C}$ for 10 min. One unit of colloidal Pt activity was defined as release of $1\text{ }\mu\text{mol H}_2$ per min.

The UV-vis absorption spectra of Mg and Zn chlorophyll-*a* were measured by a spectrophotometer (Multispec-1500 Shimadzu). The molar coefficient at the absorption maximum of Zn chlorophyll-*a* were determined by the linear plot of absorbance versus Zn chlorophyll-*a* concentration. The fluorescence emission spectrum of Zn chlorophyll-*a* was measured using a spectrofluorophotometer with a 150 W xenon lamp as a visible excitation light source (RF-5300PC Simadzu). The excitation and emission band-passes were 5.0 nm, respectively. The excitation wavelength was 600 nm. In these experiments, the absorbance at the excitation wavelength was kept constant at 0.2 for all the sample solutions.

Redox potential of chlorophyll-*a* at the ground state was measured by cyclic voltammetry. Electrolyte solution contained CH_3CN and $[n\text{-Bu}_4\text{N}] PF_6$. Energy levels of the first excited singlet state were calculated by the absorption maximum and the fluorescence maximum of Zn chlorophyll-*a*.

Photostability of Mg and Zn chlorophyll-*a* under various pH was studied using absorbance changes at the absorption of Zn and Mg chlorophyll-*a*. The sample solution consists of chlorophyll-*a* in 3 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH 4.0-8.0). The sample solution was deaerated by repeated freeze-pump-thaw cycle six times and irradiated with a 200 W tungsten lamp at a distance of 3.0 cm, with a light intensity of $200\text{ Jm}^{-2}\text{s}^{-1}$, at $30\text{ }^\circ\text{C}$. The light of the wavelength less than 390 nm was removed by a Toshiba L-39 cutoff filter.

Photoreduction of MV^{2+} was tested in the reaction mixture containing sucrose, invertase, NAD^+ , GDH, MV^{2+} and Zn chlorophyll-*a*. The reaction system consisted of sucrose (0.3 mmol), invertase (4.0 units), NAD^+ (15 μmol), GDH (5.0 units), MV^{2+} (1.2 μmol) and Zn chlorophyll-*a* (50 nmol) in 3 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH 7.0). The sample solution was deaerated by repeated freeze-pump-thaw cycle and irradiated with a 200 W tungsten lamp. The reduction of MV^{2+} was monitored using a UV-vis spectrophotometer at 605 nm, with the molar extinction coefficient of $1.3 \times 10^4\text{ mol dm}^{-3}\text{ cm}^{-1}$.

The photoinduced H_2 production from sucrose was carried out as follows. The sample solution containing sucrose (0.30 mmol), invertase (4.0 units), NAD^+ (15 μmol), GDH (5.0 units), MV^{2+} (1.2 μmol), Zn chlorophyll-*a* (50 nmol) and colloidal Pt (0.5 units) in 3 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH 7.0) was deaerated by repeated freeze-pump-thaw cycles, and substituted by Ar gas. The amount of evolved H_2 was measured by a Shimadzu GC-14B gas chromatograph (detector: TCD, column temperature: $40\text{ }^\circ\text{C}$, column: active charcoal with the particle size 60-80 mesh, carrier gas: nitrogen gas, carrier gas flow rate: 24 mL min^{-1}).

3. RESULTS AND DISCUSSION

Figure 2 shows the UV-vis absorption spectra of Mg and Zn chlorophyll-*a* in methanol solution. The absorption bands of Zn chlorophyll-*a* were 421 nm attributed to Soret band and 662 nm attributed to Q-band. On the other hand, the bands of Mg chlorophyll-*a* were 433 nm attributed to Soret band and 668 nm attributed to Q-band. In comparison of the absorption spectra of Zn and Mg chlorophyll-*a*, a blue-shift in the absorption bands of Zn chlorophyll-*a* was observed.

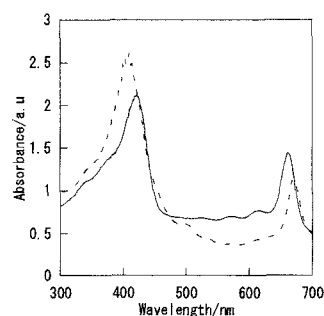


Fig. 2 UV-vis absorption spectra of Mg (dashed line) and Zn chlorophyll-*a* (solid line) in methanol solution.

Figure 3 shows fluorescence emission spectra of Zn and Mg chlorophyll-*a* in methanol solution. The fluorescence emission peak of Zn and Mg chlorophyll-*a* were 664 and 668 nm, respectively. In comparison of the fluorescence emission peak of Zn and Mg chlorophyll-*a*, the blue-shift in the emission peak of Zn chlorophyll-*a* was observed as well as that in the absorption spectrum.

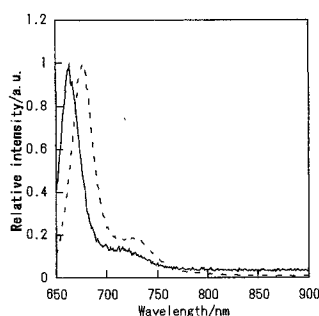


Fig.3 Fluorescence emission spectra of Zn (solid line) and Mg chlorophyll-*a* (dashed line). The excitation wavelength was 600 nm. The concentration of Zn and Mg chlorophyll-*a* were 0.6 and 0.4 $\mu\text{mol dm}^{-3}$, respectively.

Table I shows redox potential of the excited and ground state of Mg and Zn chlorophyll-*a* using cyclic voltammetry. Redox potential of Zn chlorophyll-*a* at excited state was different those of Mg chlorophyll-*a*. Zn chlorophyll-*a* is stable against irradiation. Thus, Zn chlorophyll-*a* is suitable for photosystem compared with Mg chlorophyll-*a*.

Table I. Energy levels and redox potential of the excited and ground state of Mg and Zn chlorophyll-*a*.

	[E (P ⁺ /P)] /eV	[E (P/P ⁻)] /eV	[E (P/P ⁺)] /eV	[E (P ⁺ /P ⁺)] /eV	[E (P ⁻ /P ⁻)] /eV
Mg chl- <i>a</i>	1.20	-1.27	1.84	-0.58	0.61
Zn chl- <i>a</i>	1.26	-1.23	1.87	-0.67	0.60

P⁺, P⁻ and P^{*} are potentials of one-electron oxidation, one-electron reduction and the excited state of Chl-*a*, respectively.

Photostability of Zn chlorophyll-*a* under various (pH 4.0-8.0) was investigated. The photostability was tested by irradiation with visible light using a 200 W tungsten lamp (light intensity of 200 $\text{J m}^{-2} \text{s}^{-1}$). Figure 4 shows the ratio of Mg (closed triangle) and Zn chlorophyll-*a* (closed circle) decomposition by steady irradiation under various pH conditions. The sample solution contained Zn chlorophyll-*a* (50 nmol) in 3.0 mL of 10 mmol dm^{-3} potassium phosphate buffer

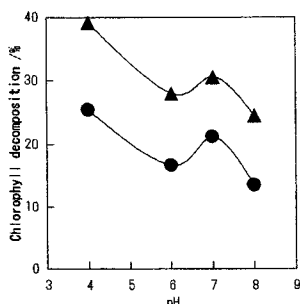


Fig.4 The ratio of Mg and Zn chlorophyll-*a* decomposition by steady irradiation under various pH. (Closed circle): The sample solution consisting of Zn chlorophyll-*a* (50 nmol) in 3.0 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH=4.0-8.0). (Closed triangle): Mg chlorophyll-*a* (50 nmol) was used instead of Zn chlorophyll-*a*.

(pH=4.0-8.0). The decomposition of Zn chlorophyll-*a* was suppressed under various pH conditions. Especially, the decomposition of Zn chlorophyll-*a* was strongly suppressed compared with Mg chlorophyll-*a* under acidic conditions (pH=4.0). Thus, Zn chlorophyll-*a* was superior to Mg chlorophyll-*a* in photostability in all pH conditions.

Figure 5 shows the time dependence of the reduced MV^{2+} ($\text{MV}^{\cdot+}$) concentration in the sample solution containing sucrose, invertase, GDH, NAD^+ , Zn chlorophyll-*a* and MV^{2+} under steady irradiation with visible light using 200 W tungsten lamp with a light intensity of 200 $\text{J m}^{-2} \text{s}^{-1}$. After 60 min irradiation, 90 $\mu\text{mol dm}^{-3}$ of reduced MV^{2+} was produced using Zn chlorophyll-*a* (closed circle), and the yield of MV^{2+} to $\text{MV}^{\cdot+}$ was estimated to be ca. 58%. The photoreduction rate was independent of the concentration of sucrose, invertase, GDH and NAD^+ . On the other hands, the reduction rate depends on the concentrations of Zn chlorophyll-*a* and MV^{2+} . Thus, the rate-limiting step in the MV^{2+} reduction is the photoinduced electron-transfer process from the photoexcited Zn chlorophyll-*a* (Zn chl-*a*^{*}) to MV^{2+} . On the other hands, MV^{2+} was not reduced without NAD^+ in the above system. There is no direct electron transfer between sucrose or glucose formed with invertase and MV^{2+} , and Zn chlorophyll-*a* and MV^{2+} . Thus, the visible light-induced MV^{2+} reduction proceeded by coupling the sucrose hydrolysis with invertase and GDH, and MV^{2+} reduction using the photosensitization of Zn chlorophyll-*a*.

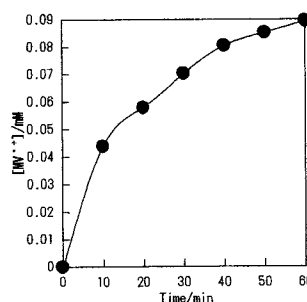


Fig.5 The time dependence of the reduced methyl viologen ($\text{MV}^{\cdot+}$) concentration under steady irradiation with visible light using 200 W tungsten lamp at a distance of 3.0 cm (light intensity of 200 $\text{J m}^{-2} \text{s}^{-1}$). The sample solution consisting of sucrose (0.30 mmol), invertase (4.0 units), GDH (5 units), NAD^+ (15 μmol), Zn chlorophyll-*a* (50 nmol) and MV^{2+} (1.2 μmol) in 3.0 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH=7.0).

As the MV^{2+} photoreduction system containing sucrose, invertase, GDH, NAD^+ , Zn chlorophyll-*a* and MV^{2+} was achieved, the photoinduced H_2 production system was investigated. Figure 6 shows the time dependence of the photoinduced H_2 production in the system containing sucrose, invertase, GDH, NAD^+ , Zn chlorophyll-*a*, MV^{2+} and colloidal Pt by visible light. By irradiation, H_2 produced continuously over more than 240 min. The amount of H_2 production was estimated to be 7.2 μmol after 240 min irradiation. It indicated that 14 μmol of proton, that was 3111 times the amount of

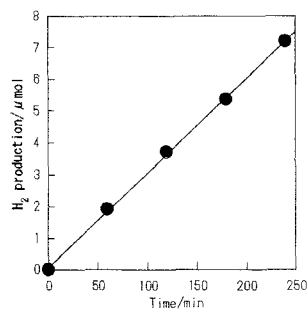


Fig.6 The time dependence of H₂ production under steady-state irradiation with visible light using 200 W tungsten lamp at a distance of 3.0 cm (light intensity of 200 Jm⁻²s⁻¹). The sample solution consisting of sucrose (0.30 mmol), invertase (4.0 units), GDH (5 units), NAD⁺ (15 μmol), Zn chlorophyll-*a* (50 nmol), MV²⁺ (1.2 μmol) and colloidal Pt (0.5 units) in 3.0 mL of 10 mmol dm⁻³ potassium phosphate buffer (pH=7.0).

Zn chlorophyll-*a* (50 nmol) in the sample solution, was reduced to H₂ molecules.

We previously reported that the decomposition of chlorophyll-*a* was suppressed by adding NAD⁺ in the system [4]. Thus, the photostability of Zn chlorophyll-*a* is improved in photoinduced H₂ production system by the addition of NAD⁺. We also previously reported the photoinduced H₂ production from methyl cellulose using the photosensitization of Mg chlorophyll-*a*. The amount of H₂ production was estimated to be 26 μmol after 420 min irradiation [22]. Since the photostability of Zn chlorophyll-*a* is superior to that of Mg Chlorophyll-*a*, steady H₂ production will be attend more than 420 min irradiation in the present system. More effective photoinduced H₂ production system will be developed by following methods.

- 1; Improvement of turn over efficiency of NAD⁺.
- 2; Development of photoinduced H₂ production system under lower pH condition with high H⁺ concentration using highly photosensitization activity of Zn chlorophyll-*a*.

4. CONCLUSION

In this work, H₂ production system from sucrose using the photosensitization of Zn chlorophyll-*a* by visible light was developed. Photostability of Mg and Zn chlorophyll-*a* under various pH conditions was investigated. The decomposition of Zn and Mg chlorophyll-*a* were 26 and 40 %, respectively under acidic conditions (pH=4.0). Thus, Zn chlorophyll-*a* was superior to Mg chlorophyll-*a* in photostability. Zn chlorophyll-*a* is suitable for photosensitizer in H₂ production system under lower pH conditions that has much proton. In H₂ production system, amount of H₂ production using Zn chlorophyll-*a* were estimated to be 7.2 μmol. Further research will be directed toward a photoinduced H₂ production system under acidic conditions using photosensitization of Zn chlorophyll-*a*.

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