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Visible and near-IR light induced biohydrogen production using the system containing Mg chlorophyll-*a* from *Spirulina* and colloidal platinum

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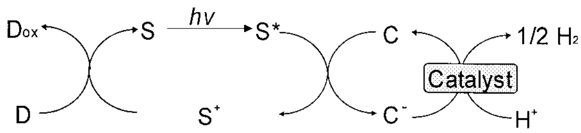
Abstract

Photoinduced hydrogen production with Mg chlorophyll-*a* from *Spirulina* as a visible and near-IR light photosensitizer by use of three component system consisting of nicotineamide adenine dinucleotide phosphate, reduced form (NADPH) as an electron donor, methylviologen as electron relay reagent and colloidal platinum as hydrogen production catalyst was investigated. After 4 h irradiation, the amount of hydrogen production with Mg chlorophyll-*a* and MgTPP, which was artificial model compound for chlorophyll, were *c.a.* 2.7 and 1.8 μmol, respectively. When the near-IR light was irradiated, little change of hydrogen production was observed. Thus, the effective visible and near IR light induced hydrogen production system with colloidal platinum was established using Mg chlorophyll-*a*.

Introduction

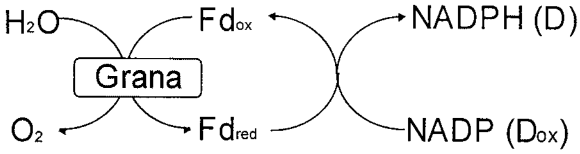
Photoinduced hydrogen production systems have been studied extensively by means of converting solar energy to chemical energy (Darwent *et al.* 1982). Photoinduced hydrogen production systems consisting of an electron donor (D), a photosensitizer (S), an electron relay (C), and a hydrogen production catalyst have been widely studied as shown in Scheme 1. For hydrogen production catalyst, colloidal platinum (Grätzel *et al.* 1979) and hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) (Okura 1985, 1986) are widely used in hydrogen production systems. Especially, colloidal platinum is stable against long-term irradiation. In photoinduced hydrogen production system with visible light, water-soluble zinc porphyrins have been widely used as effective photosensitizers, for these porphyrins have absorption band in the visible light region (380 ~ 600 nm) (Okura *et al.* 1985). However, the molar absorption coefficient of zinc porphyrins in the visible light region (500 ~ 600 nm) was 10 times lower than that in the near ultra-visible light region (380 ~ 400 nm). On the other hand, Mg chlorophyll-*a*, which acts as the effective photosensitizer in photosynthesis of green plant,



Scheme 1. Photoinduced hydrogen production system.

has absorption maximum in 432 and 670 nm (Scheer. 1991). Mg chlorophyll-*a* exhibits physiological functions as follows; the photolysis of water, the reduction of $NADP^+$ and carbon dioxide fixation under visible and near-IR light irradiation. Thus, Mg chlorophyll-*a* is attractive compound as a visible and near-IR photosensitizer for the photoinduced hydrogen production system. Photoinduced hydrogen production with modified chlorophyll and hydrogenase was reported previously (Itoh *et al.* 1998, 2000). However effective hydrogen production with Mg chlorophyll-*a* has not been reported.

Moreover, materials such as triethanolamine (TEOA) and ethylenediamine tetraacetic acid (EDTA) are sacrificial and are consumed when the photoreduction of water is carried out. Unlike these sacrificial reagents, NADPH can be a non-sacrificial electron donor. As shown in Scheme 2, oxidized NADPH



Scheme 2. Photoinduced oxygen production with grana-ferrodoxin (Fd)-NADP.

(NADP, or D_{ox}) is easily photoreduced in the presence of grana, obtained from green plants. By combining reactions in Schemes 1 and 2 the splitting of water into hydrogen and oxygen is accomplished. Thus, NADPH was useful as an electron-donating reagent in the photoinduced hydrogen production system.

In this paper we describe the photoinduced hydrogen production system with Mg chlorophyll-*a* form *Spirulina* as an effective photosensitizer in visible and near-IR region and colloidal platinum as hydrogen production catalyst and the effect of wavelength of light source on the rate of photoinduced hydrogen production with Mg chlorophyll-*a*.

Materials and methods

Reagents

Mg chlorophyll-*a* from *Spirulina* was obtained from Wako Chemical Co. Ltd (Osaka, Japan). NADPH was obtained from Oriental Yeast Co. Ltd (Tokyo, Japan). Methylviologen dichloride, cetyltrimethylammonium bromide (CTAB) and Triton X-100 were punched from Tokyo Kasei Co. Lid (Tokyo, Japan). Hydrogen hexachloplatinate hexahydrate and sodium citrate dihydrate were obtained from Kanto Chemical Co. Ltd (Tokyo, Japan). All the other reagents were higher grade available. As Mg chlorophyll-*a* is insoluble in water, 10 mmol dm^{-3} of CTAB solubilized Mg chlorophyll-*a* solution is used for the experiments.

Synthesis of magnesium tetraphenylporphyrin (MgTPP)

Magnesium tetraphenylporphyrin (MgTPP) was synthesized by refluxing TPP with an excess magnesium chloride in DMF at 60 °C for 24 h. The reaction was monitored using UV-vis spectra. During synthesis of MgTPP the initial absorption bands at 400 (Soret band), 498, 530, 568 and 622 nm (Q band) were shifted and disappeared and new bands appeared at 449 (Soret band), 550 and 610 nm (Q band). After the mixture was cooled to room temperature, MgTPP was precipitated in water. Purification of MgTPP was conducted by repeated precipitation and recrystallization from DMF-water (1:5) solution.

Preparation of colloidal platinum

Colloidal platinum was prepared with reduction of hexachloplatinate solution by sodium citrate. The reduction procedure was similar to the previously reported method (Grätzel *et al.* 1981). A solution of 400 ml of water containing 30 mg of hydrogen hexachloplatinate hexahydrate was brought to boiling temperature and then a solution of 30 ml of water containing 600 mg of sodium citrate dihydrate was added and refluxed at 100 °C for 4 h. The concentration of colloidal platinum was determined by the absorption at 400 nm with the molar coefficient (2.3×10^3 mol dm^3 cm^{-1}) using Shimadzu Multispec-1500 spectrophotometer (Kyoto Japan).

Photoreduction of methylviologen

The sample solution containing Mg chlorophyll-*a*, methylviologen and NADPH in 50 mmol dm^{-3} Tris-HCl buffer solution (pH = 7.4) was deaerated by repeated freeze-pump-thaw cycles for 6 times. The reaction volume was 3.0 ml. A Philips KP-8 200W tungsten lamp at a distance of 3.0 cm (light intensity of 200 J $m^{-2}s^{-1}$) (Tokyo, Japan) was used as steady state light source. The light of the wavelength less than 390 nm was removed by Toshiba L-39 cut-off filter. The concentration of reduced methylviologen was determined by absorption at 605 nm with the molar coefficient (1.3×104 mol dm^3 cm^{-1}) using spectrophotometer (Watanabe and Honda 1982).

Photoinduced hydrogen production with colloidal platinum

The sample solution containing Mg chlorophyll-*a*, methylviologen, NADPH and colloidal platinum in 50 mmol dm^{-3} Tris-HCl buffer solution (pH = 7.4) was deaerated by repeated freeze-pump-thaw cycles for 6 times and then substituted by argon gas for 5 min. The reaction volume was 3.0 ml. The experimental setup for the steady state irradiation was used for the photoreduction of methylviologen by Mg chlorophyll-*a*. The amount of hydrogen production was detected by Shimadzu GP-14B gas chromatography with TCD detector and active carbon column.

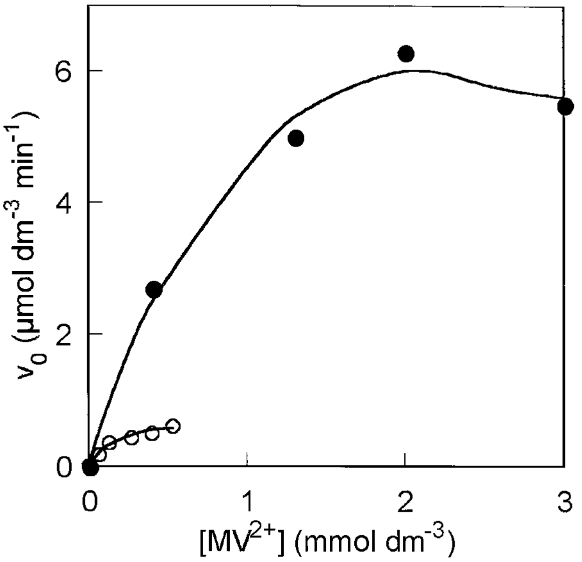


Fig 1. Methylviologen concentration versus the initial rate of methylviologen photoreduction. The sample solution consists of Mg chlorophyll-*a* or MgTPP, NADPH (2.0 mmol dm^{-3}) and methylviologen in 50 mmol dm^{-3} Tris-HCl buffer (pH = 7.4). Closed circle : Mg chlorophyll-*a* (9.0 μmol dm^{-3}); open circle: MgTPP (2.0 μmol dm^{-3}).

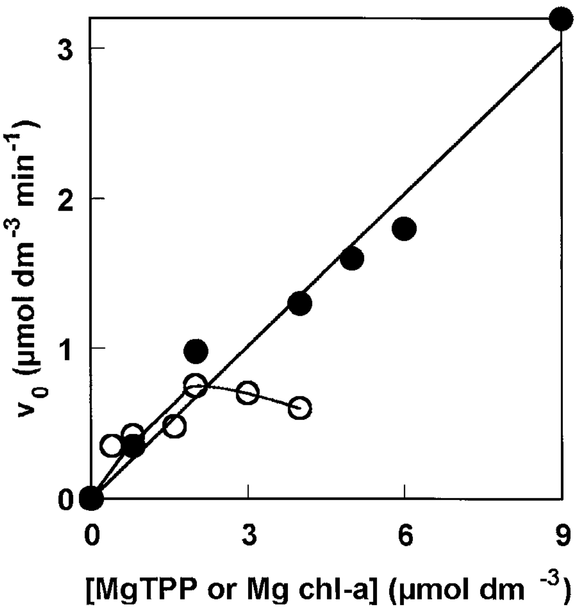


Fig 2. Photosensitizer concentration versus the initial rate of methylviologen photoreduction. The sample solution contains Mg chlorophyll-*a* or MgTPP, NADPH (2.0 mmol dm^{-3}) and methylviologen in 50 mmol dm^{-3} Tris-HCl buffer (pH = 7.4). Closed circle: Mg chlorophyll-*a* and methylviologen (2.0 mmol dm^{-3}); open circle: MgTPP and methylviologen (0.53 mmol dm^{-3}).

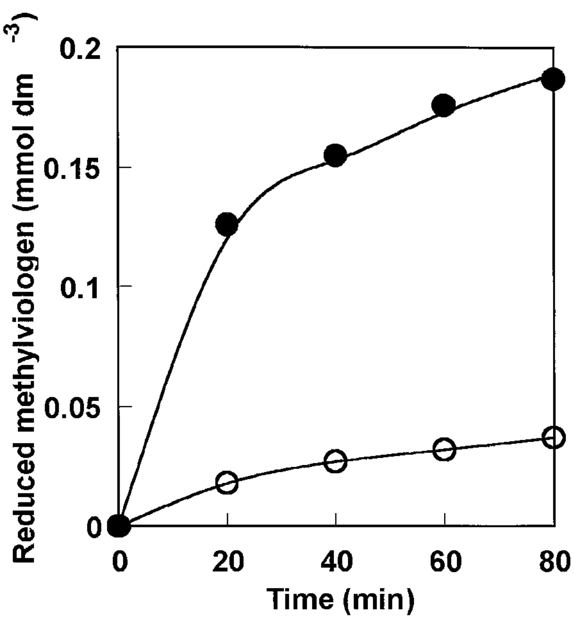


Fig 3. The time dependence of the reduced methylviologen concentration under steady state irradiation. Open circle: the sample solution consists of NADPH (2.0 mmol dm^{-3}), Mg chlorophyll-*a* (9.0 μmol dm^{-3}), and methylviologen (2.0 mmol dm^{-3}) in 50 mmol dm^{-3} Tris-HCl buffer (pH = 7.4). Closed circle: the sample solution consists of NADPH (2.0 mmol dm^{-3}), Mg TPP (2.0 μmol dm^{-3}), and methylviologen (0.53 mmol dm^{-3}) in 50 mmol dm^{-3} Tris-HCl buffer (pH = 7.4).

Results and discussion

Photoreduction of methylviologen by photosensitization of Mg chlorophyll-a

Photoreduction of methylviologen was most important step in photoinduced hydrogen production system. To attain the high yield of the reduced methylviologen, the reaction conditions of photoreduction of methylviologen consisting of NADPH, Mg chlorophyll-*a* and methylviologen were investigated. Figure 1 shows the effect of Mg chlorophyll-*a* (closed circle) and MgTPP (open circle) concentration on the initial rate (v_0) of methylviologen reduction. The initial rate was determined by the amount of reduced methylviologen with irradiation for 20 min. The reduction rate of methylviologen increased with the Mg chlorophyll-*a* concentration up to 9.0 μmol dm^{-3} and with the MgTPP concentration up to 2.0 μmol dm^{-3}.

Next let us focus on the effect of the methylviologen concentration on the initial rate. The reduction rate increased with the methylviologen concentration up to 2.0 mmol dm^{-3} for Mg chlorophyll-*a* and 0.53 mmol dm^{-3} for MgTPP as shown in Figure 2. Thus, the optimum concentrations of Mg chlorophyll-*a* and methylviologen in the methylviologen photoreduction with Mg chlorophyll-*a* were

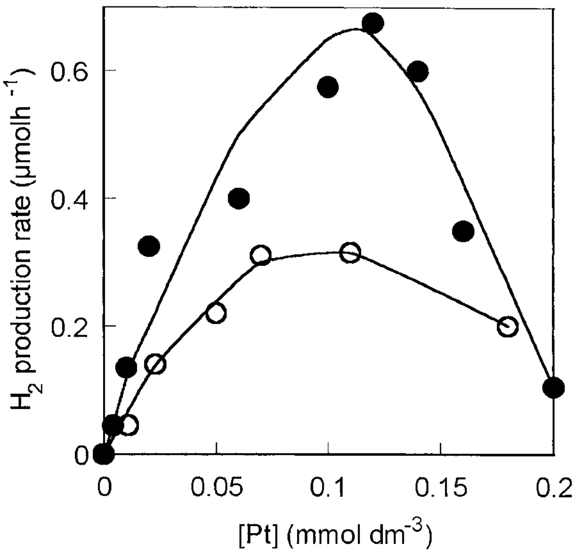


Fig 4. Colloidal platinum concentration versus the hydrogen production rate. The sample solution consists of NADPH (2.0 mmol dm^{-3}), Mg chlorophyll-*a* or MgTPP, methylviologen and colloidal platinum in 50 mmol dm^{-3} Tris-HCl buffer (pH = 7.4). Closed circle: Mg chlorophyll-*a* (9.0 μmol dm^{-3}) and methylviologen (2.0 mmol dm^{-3}); open circle: MgTPP (2.0 μmol dm^{-3}) and methylviologen (0.53 mmol dm^{-3}).

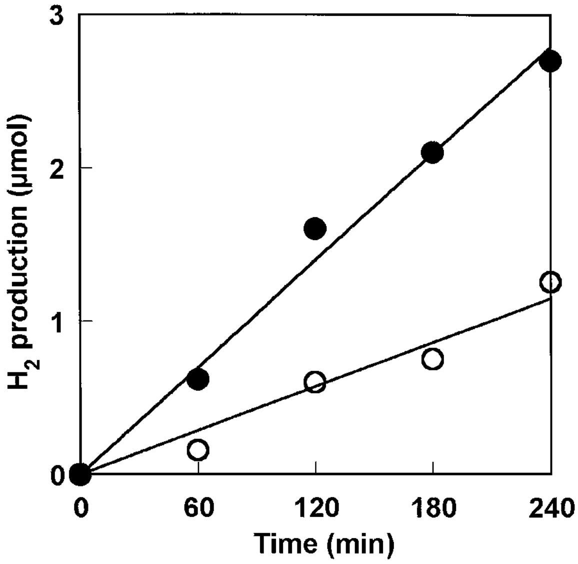


Fig 5. Time dependence of hydrogen production under steady state irradiation. The sample solution consists of NADPH (2.0 mmol dm^{-3}), Mg chlorophyll-*a* or MgTPP, methylviologen, and colloidal platinum (20 μmol dm^{-3}) in Tris-HCl buffer (pH = 7.4). Closed circle: Mg chlorophyll-*a* (9.0 μmol dm^{-3}) and methylviologen (2.0 mmol dm^{-3}); open circle: MgTPP (2.0 μmol dm^{-3}) and methylviologen (0.53 mmol dm^{-3}).

9.0 μmol dm^{-3} and 2.0 mmol dm^{-3}, respectively. On the other hand, optimum concentrations of MgTPP and methylviologen in the methylviologen photoreduction with MgTPP were 2.0 μmol dm^{-3} and 0.53 mmol dm^{-3}, respectively. When the optimum conditions with NADPH (2.0 mmol dm^{-3}) in Tris-HCl buffer (pH = 7.4) was irradiated at 30 °C, the accumulation of reduced methylviologen was observed as shown in Figure 3. After 80 min irradiation, the reduced methylviologen concentration was 0.19 mmol dm^{-3}. The reduction ratio of methylviologen was 10% and the turnover number of Mg chlorophyll-*a* was estimated to be 0.70 min^{-1}. In the case of the system using MgTPP under the same reaction condition, on the other hand, the reduction ratio of methylviologen and the turnover number of MgTPP was estimated to be 8.0% and 0.09 min^{-1}, respectively. Thus, the effective photoreduction system was accomplished using Mg chlorophyll-*a*.

Photoinduced hydrogen production with Mg chlorophyll-a and colloidal platinum

As the effective photoreduction system was accomplished, the photoinduced hydrogen production with colloidal platinum was attempted under above conditions. The effect of colloidal platinum concentration on the hydrogen production rate was investigated. The rate was determined by the amount of hydrogen production with irradiation for 240 min. In the cases of the systems with Mg chlorophyll-*a* and with MgTPP, the hydrogen production rate increased with the colloidal platinum concentration up to 0.12 mmol dm^{-3} and then decreased through the maximum value as shown in Figure 4. Thus, the optimum concentration of colloidal platinum was 0.12 mmol dm^{-3}. There are two reasons in the rate decrease at higher colloidal platinum concentration. As the aggregation of colloidal platinum may take place at higher concentration of colloidal platinum (> 0.15 mmol dm^{-3}), the hydrogen production activity of colloidal platinum may decrease. The other reason is explained as follows. At higher concentration of colloidal platinum, the filter effect of aggregation of colloidal platinum may occur and hydrogen production rate may decrease.

When the sample solution containing Mg chloro phyll-*a* or MgTPP, methylviologen, NADPH (2.0 mmol dm^{-3}) and colloidal platinum (0.12 mmol dm^{-3}) in Tris-HCl buffer (pH = 7.4) was irradiated at 30 °C, hydrogen production was observed as shown in Figure 5. The amount of hydrogen production increased with irradiation time. After 4 h irradiation, the

Table 1. The rates of methylviologen photoreduction and photoinduced hydrogen production with Mg chlorophyll-*a* and MgTPP under the irradiation through the IR transmittance filter.

	Without filter		IR transmittance filter	
	MV^{2+} reduction rate (μmoldm^{-3} min^{-1})	Hydrogen production rate (μmol h^{-1})	MV^{2+} reduction rate (μmoldm^{-3} min^{-1})	Hydrogen production rate (μmol h^{-1})
Mg Chl-*a*	0.36	0.68	0.42	0.65
MgTPP	0.46	0.33	0.28	0.20

MV^{2+}: methylviologen, Mg Chl-*a*: Mg chlorophyll-*a*
Reaction conditions: The sample solution consists of NADPH (2.0 mmol dm^{-3}), Mg chlorophyll-*a* (9.0 μmol dm^{-3}) or MgTPP (2.0 μmol dm^{-3}), and methylviologen (2.0 mmol dm^{-3}) in Tris-HCl buffer (pH = 7.4). For hydrogen production, the colloidal platinum (20 μmol dm^{-3}) was added to above solution.

amount of hydrogen production with Mg chlorophyll-*a* and with MgTPP were ca. 2.7 and 1.8 μmol, respectively. Thus, the effective hydrogen production system was accomplished using Mg chlorophyll-*a*. The effects of the above four components, NADPH, Mg chlorophyll-*a*, methylviologen and colloidal platinum, on the hydrogen production were investigated. It was found that hydrogen was not evolved even in the missing of one of the above four components, NADPH, Mg chlorophyll-*a*, methylviologen and colloidal platinum.

Effect of wavelength of light source on the rates of methylviologen photoreduction and photoinduced hydrogen production

The effect of wavelength of light source on the rates of methylviologen photoreduction and photoinduced hydrogen production with Mg chlorophyll-*a* and MgTPP were investigated. The rates of methylviologen photoreduction and photoinduced hydrogen production changes with 200 W tungsten lamp through the IR transmittance filter were shown in Table 1. The IR transmittance filter transmits the light of the wavelength between 600 and 740 nm. For the system using Mg chlorophyll-*a*, little change in the rates of methylviologen photoreduction and photoinduced hydrogen production were observed with the irradiation through the IR transmittance filter compared with that of the irradiation without optical color filter. For the system using MgTPP, on the other hand, the rates of methylviologen photoreduction and photoinduced hydrogen production decreased with the irradiation through the IR transmittance filter. Mg chlorophyll-*a* has absorption maxima at 450, 630 and 663 nm and the molar coefficient at 663 nm is 4.1 × 104 mol dm^3 cm^{-1}. On the other hand, Mg TPP has absorption maxima at 449, 550 and 610 nm and the molar coefficient at 610 nm is 1.0 × 104 mol dm^3 cm^{-1}. Mg chlorophyll-*a* effectively absorbed near IR light compared with MgTPP. Thus, the effective visible and near IR light induced methylviologen reduction and hydrogen production system with colloidal platinum were established using the photosensitization of Mg chlorophyll-*a*.

Conclusion

In this work, photoinduced hydrogen production with Mg chlorophyll-*a* from *Spirulina* as a visible and near-IR light photosensitizer by use of three component system consisting of NADPH, methylviologen and colloidal platinum was investigated. The effective visible and near IR light induced methylviologen reduction and hydrogen production system with colloidal platinum were established using the photosensitization of Mg chlorophyll-*a*.

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