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Visible light-induced enzymatic hydrogen production from oligosaccharides using Mg chlorophyll-*a* and platinum colloid conjugate system

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Abstract

Visible light-induced enzymatic hydrogen production coupling the enzymatic oligosaccharide degradation and hydrogen production with platinum colloid using the photosensitization of Mg chlorophyll-*a* (Mg Chl-*a*) has been developed. The continuous hydrogen gas production was observed when the reaction mixture containing oligosaccharide (sucrose or maltose), invertase, glucose dehydrogenase, nicotinamide adenine dinucleotide (NAD^+), Mg Chl-*a*, methylviologen (MV^{2+}, an electron relay reagent) and platinum colloid was irradiated by visible light. After 420 min irradiation, the amounts of hydrogen production from sucrose and from maltose were estimated to be 4.3 and 0.40 μmol, respectively.
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Keywords: Platinum colloid; Mg chlorophyll-*a*; Biomass; Oligosaccharide; Hydrogen production

1. Introduction

Enzymatic hydrogen production from the renewable biomass resources including starch, sucrose and lactose is important in the environmental and the development of energy source research fields [1–5]. These resources are oligosaccharides or polysaccharides and are hydrolyzed to form monosaccharides such as glucose and fructose. Some studies on the hydrogen production from glucose using the enzymatic pathway have been reported [6–10]. The hydrogen production from glucose with the combination of glucose dehydrogenase (GDH) and hydrogenase has been reported [11,12]. However, the enzymatic photoinduced hydrogen production from oligosaccharides or polysaccharides has been paid little attention. Glucose was obtained from sucrose by using invertase enzymatically. Thus, the hydrogen production from oligosaccharide, sucrose will be attained using the combination of invertase, GDH and hydrogenase or the other hydrogen evolved catalyst.

On the other hands, some photoinduced hydrogen production systems consist of electron donor, photosensitizer, electron carrier and catalyst for hydrogen production [13–19]. For hydrogen evolved catalyst, platinum colloid [17–19] and hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) [13–16] are widely used in hydrogen production systems. Especially, platinum colloid is stable against long-term irradiation. In photoinduced hydrogen production system, to use the visible light effectively, highly active photosensitizer is desired. Mg chlorophyll-*a* (Mg Chl-*a*), which acts as the effective photosensitizer in photosynthesis of green plant [20], has absorption maximum at 670 nm. Thus, Mg Chl-*a* is attractive compound as a visible photosensitizer. We previously reported the photoinduced hydrogen production with the photosensitization of Mg Chl-*a* [21,22]. In photoinduced hydrogen production with the system consisting of an electron donor, photosensitizer, electron relay and catalyst, photoexcited photosensitizer reacts with electron relay to form the reduced electron relay and hydrogen evolves by the proton reduction with the catalyst and then the oxidized photosensitizer is reduced by electron-donating reagent such as reduced nicotinamide adenine dinucleotide (NADH). Thus, the electron donor,

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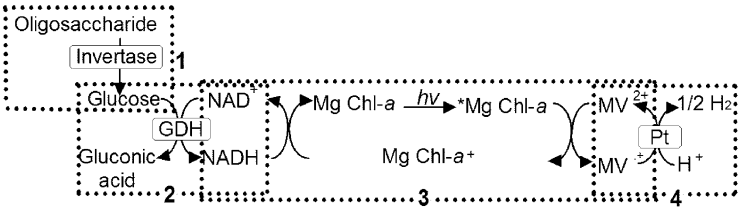

Scheme 1. Visible light-induced enzymatic hydrogen production system coupling the oligosaccharide degradation with invertase and GDH and hydrogen production with platinum colloid using the photosensitization of Mg Chl-*a* in the presence of MV^{2+}.

NADH was sacrificial reagent and the oxidized electron donor, NAD^+ was consumed in the reaction system. If NADH is regenerated, the photoinduced hydrogen production system is accomplished without NAD^+ consumption. As GDH uses NAD^+ as a cofactor, the photoinduced hydrogen production with GDH, electron donor, photosensitizer, electron relay reagent, and catalyst will be attained.

In this work we describe the visible light-induced hydrogen production system coupling the oligosaccharide (sucrose or maltose) degradation with invertase and GDH, and hydrogen production with platinum colloid using the photosensitization of Mg Chl-*a* in the presence of methylviologen (MV^{2+}) as an electron relay reagent as shown in Scheme 1.

2. Experimental

2.1. Materials

Mg Chl-*a* from *spirulina*, invertase from Yeast and GDH from *Bacillus* sp. were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). NAD^+ and NADH were purchased from Oriental Yeast Co. Ltd. MV^{2+} dichloride and cetyltrimethylammonium bromide (CTAB) were punched from Tokyo Kasei Co. Ltd (Tokyo, Japan). Hydrogen hexachloplatinate hexahydrate and sodium citrate dihydrate were obtained from Kanto Chemical Co. Ltd (Tokyo, Japan). 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 NAD^+ to NADH by glucose per min. One unit of invertase activity was defined as the amount of enzyme that produced 1.0 μmol glucose by sucrose per min. Mg Chl-*a* was solubilized with 10 mmol dm^{-3} of CTAB, since Mg Chl-*a* is insoluble to aqueous solution.

2.2. Preparation of platinum colloid

Platinum colloid was prepared by reduction of hexachloplatinate solution with sodium citrate [17]. A solution of 400 ml of water containing 30 mg of hydrogen hexachloplatinate hexahydrate was brought to boiling temperature using mantle heater with magnetic stirrer for 1.5 h and then a solution of 30 ml of water containing 600 mg of sodium citrate dihydrate was added and refluxed with stirrer at 100°C for 4 h. The particle size of platinum colloid prepared was estimated to be 1.5 nm by A. In general, the platinum colloid activity decreased with increase the particle size (more than 2.0 nm) [17]. The prepared platinum colloid has the ability to release 0.7 μmol of hydrogen in the reaction system of 10 μl platinum colloid, 1.2×10^{-5} mmol of MV^{2+} and 7.7×10^{-5} mmol of sodium dithionite in 4.0 ml of 50 mmol dm^{-3} Tris-HCl buffer (pH 7.4) at 30°C for 10 min. One unit of platinum colloid activity was defined as release of 1.0 μmol of hydrogen per min.

2.3. NADH formation with oligosaccharide, invertase and GDH

The reaction was started by addition of NAD^+ solution to the sample solution containing oligosaccharide (sucrose or maltose), invertase and GDH in 3.0 ml of 10 mmol dm^{-3} phosphate buffer (pH 7.0). The reduction of NAD^+ to NADH was monitored using UV–vis spectrophotometer at 340 nm, with the molar extinction coefficient of 6.3×10^3 mol^{-1} dm^3 cm^{-1}.

2.4. Photoreduction of MV^{2+}

Photoreduction of MV^{2+} was tested in the reaction mixture containing NAD^+, oligosaccharide (sucrose or maltose), Mg Chl-*a*, MV^{2+}, invertase and GDH. The reaction system consisted of NAD^+, oligosaccharide, Mg Chl-*a*, MV^{2+}, invertase and GDH in 3.0 ml of 10 mmol dm^{-3} potassium phosphate buffer (pH 7.0). The sample solution was deaerated by repeated freeze-pump-thaw cycles and irradiated with a 200 W tungsten lamp at a distance of 3.0 cm at 30°C. The light of the wavelength less than 390 nm was removed by Toshiba L-39 cut-off filter (Tokyo, Japan). The reduction of MV^{2+} was monitored using UV–vis spectrophotometer at 605 nm, with the molar extinction coefficient of 1.3×10^4 mol dm^3 cm^{-1} [23].

2.5. Visible light-induced hydrogen production

The photoinduced hydrogen production from oligosaccharide was carried out as follows. The sample solution containing NAD^+, oligosaccharide, Mg Chl-*a*, MV^{2+}, platinum

colloid, invertase and GDH in 3.0 ml of 10 mmol dm^{-3} potassium phosphate buffer (pH 7.0) was deaerated by freeze pump thaw cycle for 6 times, and substituted by argon gas. The amount of hydrogen evolved was measured by Schimadzu GC-14B gas chromatography (detector: TCD, column temperature: 40°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

3.1. NADH formation with oligosaccharide, invertase and GDH system

When the sample solution containing sucrose, invertase, GDH and NAD^+ was incubated, the time dependence of the NADH formation is shown in Fig. 1 (closed circle). The initial rate of NADH formation, which was determined by the amount of NADH with incubation for 10 min, was estimated to be 3.4×10^{-8} mol min^{-1}. After 80 min incubation, 0.77 μmol NADH was formed. The yield of the conversion of NAD^+ to NADH in this system was ca. 100%. When the sample solution containing maltose, invertase, GDH and NAD^+ was incubated, the time dependence of the NADH formation is shown in Fig. 1 (closed square). By using maltose, the initial rate was estimated to be 2.2×10^{-9} mol min^{-1}. After 80 min incubation, 0.067 μmol of NADH was formed. The yield of the conversion of NAD^+ to NADH in this system was ca. 10%. Invertase hydrolyses the terminal non-reducing β-fructofuranoside residues in oligosaccharide. Sucrose is a oligosaccharide composed of an glucose molecule and fructose molecule linked by an α-1,4-glycoside bond. Thus, sucrose is easily hydrolyzed with invertase. The Michaels–Menten constant K_m value of sucrose for invertase in phosphate buffer (pH 7.0) was estimated to be 0.78 mmol dm^{-3} by the Micheals–Menten equation. On the other hand, maltose is a oligosaccharide composed of two glucose molecules linked by an α-1,4-glycoside bond. There is no terminal non-reducing β-fructofuranoside residue in maltose. The K_m value of maltose for invertase in phosphate buffer (pH 7.0) was estimated to be 8.9 mmol dm^{-3}. These results indicated that sucrose has a high affinity for invertase compared with that of maltose. As glucose formation from sucrose (process 1 in Scheme 1) with invertase rapidly proceeded, the high yield of NADH formation was obtained [24]. This result shows that glucose formation from maltose with invertase slower proceeded due to the low affinity for invertase.

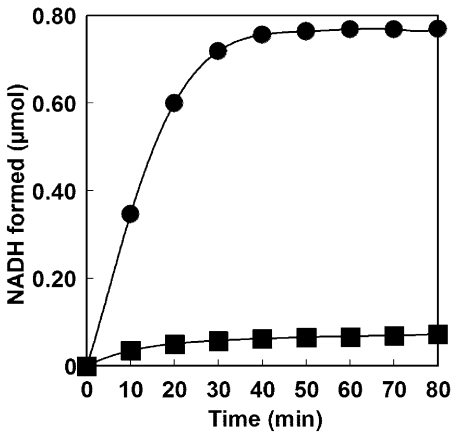

Fig. 1. Time dependence of NADH formation with oligosaccharide (sucrose or maltose 11 μmol), NAD^+ (0.77 μmol), invertase (4.0 units) and GDH (5.0 units) in 3.0 ml of 10 mmol dm^{-3} phosphate buffer (pH 7.0). Closed circle and square are sucrose and maltose, respectively.

3.2. Photoreduction of MV^{2+}

Fig. 2 shows the time dependence of the reduced MV^{2+} concentration in the system containing NAD^+, oligosaccharide, Mg Chl-*a*, MV^{2+}, invertase and GDH with visible light irradiation. The absorbance at 605 nm, absorption band of reduced MV^{2+}, increased with irradiation time. The initial rate of reduced MV^{2+} formation in the system using sucrose and maltose, which was determined by the amount of reduced MV^{2+} with irradiation for 20 min, was estimated to be 8.6×10^{-9} and 8.7×10^{-10} mol min^{-1}, respectively. After 80 min irradiation, 0.20 and 0.02 μmol of reduced MV^{2+} were produced in the system using sucrose (closed circle) and maltose (closed square) as an oligosaccharide, respectively. The yields of MV^{2+} to reduced MV^{2+} for the

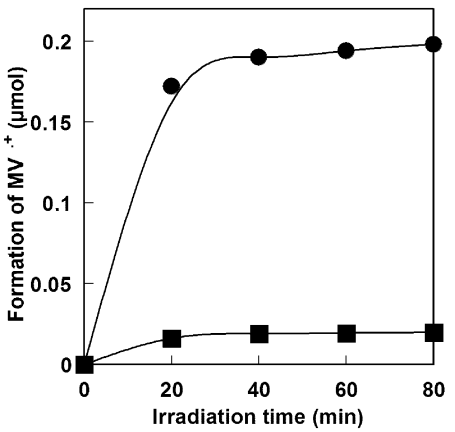

Fig. 2. The time dependence of the reduced MV^{2+} concentration under steady state irradiation with visible light using 200 W tungsten lamp at a distance of 3.0 cm (light intensity of 200 J m^{-2} s^{-1}). The sample solution consisting of NAD^+ (11 μmol), oligosaccharide (0.30 mmol), Mg Chl-*a* (3.0 nmol) and MV^{2+} (1.2 μmol), invertase (4.0 units) and GDH (5.0 units) in 3.0 ml of 10 mmol dm^{-3} phosphate buffer (pH 7.0). Closed circle and square are sucrose and maltose, respectively.

system using sucrose and maltose after 80 min irradiation were estimated to be ca. 17% and 1.7%, respectively. The lag phase in the MV^{2+} photoreduction with coupling the oligosaccharide degradation and the MV^{2+} photoreduction was not observed, indicating that NAD^+ effectively is regenerated to NADH with oligosaccharide degradation and the rate-limiting step in whole reaction is the photoreduction of MV^{2+}. On the other hand, MV^{2+} was not reduced without NAD^+ in the above system. There is no direct electron transfer between oligosaccharide or glucose formed with invertase and MV^{2+}, and between Mg Chl-*a* and MV^{2+}. Thus, the visible light-induced MV^{2+} reduction proceeded by coupling the oligosaccharide degradation with invertase and GDH (processes 1 and 2 in Scheme 1) and MV^{2+} reduction using the photosensitization of Mg Chl-*a* (process 3 in Scheme 1).

3.3. Visible light-induced hydrogen production

As the MV^{2+} photoreduction system containing NAD^+, oligosaccharide, Mg Chl-*a*, MV^{2+}, invertase and GDH was achieved, the photoinduced hydrogen production system was investigated. Fig. 3 shows the time dependence of the photoinduced hydrogen production in the system containing NAD^+, oligosaccharide, Mg Chl-*a*, MV^{2+}, platinum colloid, invertase and GDH by the visible light. By irradiation, hydrogen evolved continuously more than 420 min. After 420 min irradiation, the amount of hydrogen production from sucrose and from maltose were estimated to be 4.3 and 0.40 μmol, respectively. On the other hand, the hydrogen also was not evolved in the absence of NAD^+ in the above system. There is no direct electron transfer between sucrose or glucose formed with invertase and platinum colloid, and among Mg Chl-*a*, MV^{2+} and platinum colloid. These results suggest that the visible light-induced hydrogen production proceeded by coupling the sucrose degradation with invertase and GDH (processes 1 and 2 in Scheme 1) and the hydrogen production with platinum colloid using the photosensitization of Mg Chl-*a* (processes 3 and 4 in Scheme 1). As the hydrogen production rate was independent of platinum colloid concentration between 0.3 and 0.7 units, the rate-limiting step in whole reaction is the photoreduction of MV^{2+} and continuous hydrogen production is observed under lower MV^{2+} reduction. Inoue et al. reported the photoinduced hydrogen production from glucose with the photosensitization of water-soluble zinc porphyrin and hydrogenase as a hydrogen production catalysis [25]. In this system, 1.0 μmol hydrogen evolved after 4 h irradiation, and hydrogen evolved continuously 8 h and then saturated by continuous irradiation. After 8 h irradiation, the amount of hydrogen production was estimated to be 2.6 μmol. In contrast, hydrogen evolved continuously 24 h and then saturated by continuous irradiation in our system. After 24 h irradiation, the amount of hydrogen production from sucrose and maltose were estimated to be 12.5 and 1.80 μmol, respectively. Thus, the hydrogen production from sucrose and maltose system with Mg Chl-*a* and platinum colloid is superior to the system with zinc porphyrin and hydrogenase.

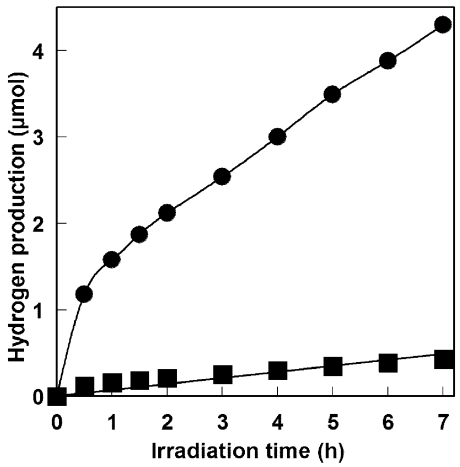

Fig. 3. Time dependence of hydrogen production under steady state irradiation with visible light using 200 W tungsten lamp at a distance of 3.0 cm (light intensity of 200 J m^{-2} s^{-1}). The sample solution consisting of invertase (4.0 units), GDH (5.0 units) NAD^+ (11 μmol), oligosaccharide (0.30 mmol), Mg Chl-*a* (3.0 nmol) and MV^{2+} (1.2 μmol), and platinum colloid (0.5 unit) in 3.0 ml of 10 mmol dm^{-3} phosphate buffer (pH 7.0). Closed circle and square are sucrose and maltose, respectively.

The effects of wavelength of light source on the amount of photoinduced hydrogen production were investigated. The amount of hydrogen production changes with 200 W tungsten lamp through the IR transmittance filter was detected. The IR transmittance filter transmits the light of the wavelength between 600 and 740 nm. After 420 min irradiation through the IR transmittance filter, the amount of hydrogen production from sucrose and maltose were estimated to be 4.0 and 0.36 μmol, respectively. Little change in the amount of hydrogen production was observed with the irradiation through the IR transmittance filter compared with that of the irradiation without optical color filter. As Mg Chl-*a* has absorption maxima at 450, 630 and 663 nm and the molar coefficient at 663 nm is 4.1×10^4 mol^{-1} dm^3 cm^{-1}, Mg Chl-*a* effectively absorbed near IR light and the effective visible and near IR light induced hydrogen production system was established.

4. Conclusion

In conclusion, hydrogen production system coupling the oligosaccharide degradation with invertase and GDH and hydrogen production with platinum colloid using the visible light-induced photosensitization of Mg Chl-*a* was developed and the continuous hydrogen gas was gained. The renewable biomass resources are effectively used to convert environmentally clean energy source, hydrogen gas.

Acknowledgements

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