Polymer Journal, Vol. 36, No. 4, pp. 352—355 (2004)

NOTES

Hydrogen Production from Cellulose Derivative with the System Containing Mg Chlorophyll-*a* and Platinum Colloid

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(Received August 14, 2003; Accepted January 5, 2004)

KEY WORDS Cellulose / Mg Chlorophyll-*a* / Hydrogen Production / Platinum Colloid / Cellulase /

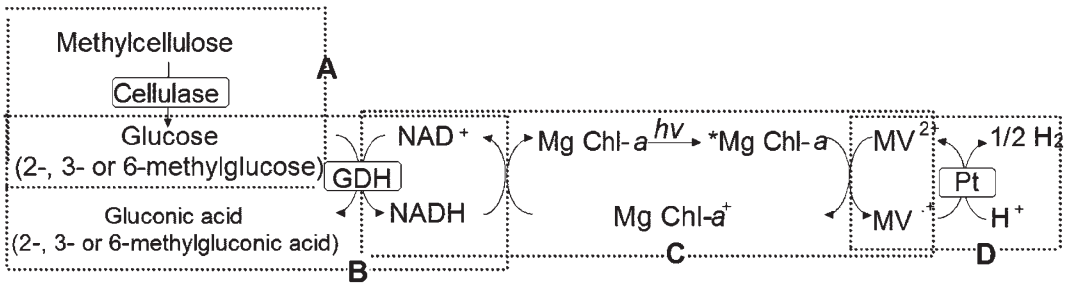
Hydrogen production system from renewable bioresources of timber waste, including cellulose and lignin, is important in energy source research fields.[1–3] Cellulose and other polysaccharides can be hydrolyzed to monosaccharides, which can then be converted to hydrogen gas. Hydrogen production from glucose with glucose dehydrogenase (GDH) and a hydrogenase has been reported.[4,5] Hydrogen production from glucose with GDH, hydrogenase and photoredox reaction with photosensitizer such as a zinc porphyrin also has been reported.[6] Enzymatic photoinduced hydrogen production from polysaccharides, such as cellulose, has not received much attention. We previously reported enzymatic photoinduced hydrogen production from the oligosaccharide, sucrose, by the photosensitization of Mg chlorophyll-*a* or artificial Zn chlorophyll-*a* with platinum colloid.[7,8] Photoinduced hydrogen production from polysaccharide, such as cellulose using the photosensitization of chlorophyll-*a*, has not been accomplished. Our investigation first focused on the development of a visible light-induced hydrogen production system by coupling cellulose from cotton hydrolysis using cellulase, with GDH and photoinduced hydrogen production with Mg chlorophyll-*a* and platinum colloid. Cellulose did not hydrolyze enough using cellulase and GDH, and no hydrogen production was observed. The degree of polymerization and molecular weight of the cellulose in the photoinduced hydrogen production system are important factors. Thus, relations among the degree of polymerization, molecular weight of cellulose and the amount of hydrogen production were examined using solubilized cellulose, methylcellulose as a cellulose model, in the photoinduced hydrogen production system.

This paper describes a hydrogen production system by coupling methylcellulose as a cellulose derivative hydrolysis using cellulase, with GDH and photoinduced hydrogen production with Mg chlorophyll-*a* and platinum colloid as shown in Scheme 1, and the effect of polymerization degree of methylcellulose on the hydrogen production rate.

EXPERIMENTAL

Materials

Mg chlorophyll-*a* from *Spilurina*, glucose dehydrogenase (GDH) from *Bacillus* sp. and methylcellulose were purchased from Wako Pure Chemical Industries, Ltd. Methylcellulose (MC_n) with average molecular weights of 15.000, 21.000, 26.000, 41.000, 63.000 and 86.000, are designated as the MC_1, MC_2, MC_3,



Scheme 1. Visible light-induced hydrogen production system coupling methylcellulose hydrolysis with cellulase and GDH and hydrogen production with platinum colloid by photosensitization of Mg chlorophyll-*a* (Mg Chl-*a*) in the presence of methylviologen (MV^{2+}).

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MC_4, MC_5 and MC_6. NAD^+ and NADH, were purchased from Oriental Yeast Co., Ltd. Cellulase from *Aspergillus niger* was obtained from Sigma. Methylviologen dichloride and cetyltrimethylammonium bromide (CTAB) were from Tokyo Kasei Co., Ltd. One unit of GDH activity was defined as the amount of enzyme that would reduce 1 μmol NAD^+ to NADH by glucose per min. One unit of cellulase activity was defined as the amount of enzyme that would produce 1 μmol glucose by MC_1 per min. As Mg chlorophyll-*a* was solubilized in 10 mmol dm^{-3} CTAB.

Preparation of Platinum Colloid

Platinum colloid was prepared by refluxing hydrogen hexachloplatinate(IV) hexahydrate and sodium citrate as previously reported.[9] The prepared platinum colloid released 0.7 μmol hydrogen in the reaction system of 10 μL platinum colloid, 120 μmol methylviologen and 770 μmol sodium dithionite in 4 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 the release of 1 μmol of hydrogen per min.

NADH Formation with Cellulase, GDH and Methylcellulose

The reaction was started by addition of NAD^+ (0.6 μmol) solution to the sample solution containing MC_n (1.2 μmol, cellobiose unit), cellulase (4.0 units) and GDH (5.0 units) in 3.0 mL of 10 mmol dm^{-3} phosphate buffer (pH = 7.0). The reduction of NAD^+ to NADH was determined with a UV–vis spectrophotometer (Multispec-1500 by Shimadzu Corp.) at 340 nm at a molar extinction coefficient of 6.3×10^3 mol dm^3 cm^{-1}.

Photoreduction of Methylviologen

A methylviologen photoreduction system containing NAD^+, MC_n, Mg chlorophyll-*a*, methylviologen, cellulase and GDH (steps A, B and C in Scheme 1) was investigated. For photolysis under steady state irradiation, a 200 W tungsten lamp was used at 30 °C. The light of the wavelength less than 390 nm was cut a L-39 cut-off filter by Toshiba Corp. Reduction in methylviologen was determined using a UV–vis spectrophotometer at 605 nm.[10]

Visible-Light Induced Hydrogen Production

Photoinduced hydrogen production from MC_n was carried out as follows. The sample solution containing MC_n (0.3 mmol cellobiose unit), Mg chlorophyll-*a* (27 nmol), methylviologen (1.2 μmol), colloidal platinum (0.5 unit), cellulase (4.0 units) and GDH (5.0 units) in 10 mmol dm^{-3} phosphate buffer (pH = 7.0) was deaerated by freeze pump thaw cycle for 6 times and substituted by argon gas. NAD^+ (10 μmol) solution, deaerated and substituted by argon gas, was added to the above solution and the reaction was started by irradiation. Reaction volume was 3.0 mL. The hydrogen produced was measured by gas chromatography (detector: TCD, column: active charcoal column, carrier gas: nitrogen).

RESULTS AND DISCUSSION

NADH Formation with Cellulase, GDH and Methylcellulose System

Methylglucose (2-, 3- or 6-methylglucose) or glucose formation from methylcellulose with cellulase was first measured. The reaction was started by cellulase (4.0 units) addition to the sample solution containing MC_n (1.2 μmol, cellobiose unit) in 3.0 mL 10 mmol dm^{-3} phosphate buffer (pH = 7.0). For all systems using MC_n, 1.2 μmol methylglucose or glucose was produced at 10 min incubation. This shows that the rate of methlycellulose hydrolysis to methylglucose or glucose with cellulase is very rapid. The yield was *ca.* 100% at 10 min incubation. When the sample solution containing MC_n, cellulase, NAD^+ and GDH was incubated, the time dependence of yield of NAD^+ to NADH is shown in Figure 1. For all systems using MC_n, 0.6 μmol NADH was formed and the yield of conversion of NAD^+ to NADH by the MC_n hydrolysis with cellulase and GDH was 100% after 40 min incubation. Little change in NADH formation rate was observed for any MC_n, and thus, MC_n hydrolysis rate with cellulase (steps A in Scheme 1) is independent of the polymerization of MC_n.

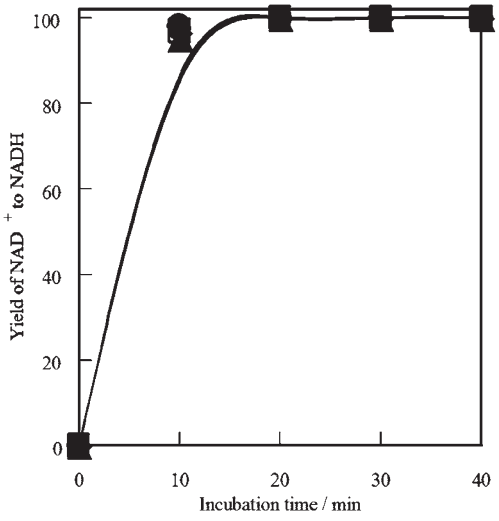

Figure 1. Time dependence of yield of NAD^+ to NADH with MC_n (1.2 μmol cellobiose unit), cellulase (4.0 units), NAD^+ (0.6 μmol) and GDH (5.0 units) in 3 mL 10 mmol dm^{-3} phosphate buffer (pH 7.0). Closed square: MC_1, closed circle: MC_2, closed triangle: MC_3, closed diamond: MC_4, open square: MC_5 and open circle: MC_6.

Methylviologen Photoreduction

When the deaerated sample solution containing NAD^+ (7.5 μmol), MC_n (0.3 mmol cellobiose unit), Mg chlorophyll-*a* (27 nmol), methylviologen (1.2 μmol), cellulase (4.0 units) and GDH (5.0 units) in 3.0 mL of 10 mmol dm^{-3} phosphate buffer (pH = 7.0) was irradiated, absorbance at 605 nm due to reduced methylviologen formation, increased with irradiation time. For all systems using MC_n, *ca.* 1.2 μmol reduced methylviologen was produced and the conversion of methylviologen to reduced methylviologen was 100% at 180 min irradiation. The methylviologen photoreduction rate was not affected by polymerization of MC_n. No reduced methylviologen formation was observed without irradiation. Thus, the visible light-induced methylviologen reduction proceeds by coupling the MC_n hydrolysis with cellulase and GDH (steps A and B in Scheme 1) and methylviologen reduction with photosensitization of Mg chlorophyll-*a* (step C in Scheme 1).

Visible-Light Induced Hydrogen Production

The methylviologen photoreduction system containing MC_n (steps A, B and C in Scheme 1) was realized and the photoinduced hydrogen production system was developed by adding platinum colloid to the methylviologen photoreduction system. When the sample solution was irradiated, hydrogen production was observed as shown in Figure 2. For all systems using MC_n, hydrogen produced more than 4 h, hydrogen production was above 10 μmol after 4 h irradiation. For systems using MC_1 and MC_4, hydrogen production was 12 μmol. Hydrogen production was 10 μmol in the system using MC_2. Hydrogen production rate is thus independent of polymerization of MC_n. For all systems using MC_n, quantum yield was *ca.* 2.0% by the potassium ferrioxalate actinometry method.[11] In all cases, hydrogen production was larger than that of the initial NAD^+ (7.5 μmol). This indicates that NADH formed by cellulase and GDH is oxidized to NAD^+ in photoinduced hydrogen production and NAD^+ is reduced to NADH by MC_n hydrolysis with cellulase and GDH, and then NADH serves as electron donor in photoinduced hydrogen production. No hydrogen was produced in the absence of NAD^+ in the above system. No hydrogen production was observed without irradiation as shown in Figure 2. Visible light-induced hydrogen production may thus proceed by coupling MC_n hydrolysis with cellulase and GDH (steps A and B in Scheme 1) and hydrogen production with platinum colloid using the photosensitization of Mg chlorophyll-*a* (steps C and D in Scheme 1). Steady hydrogen production was observed at over 8 h irradiation. Product inhibition and deactivation of enzymes hardly occur in this reaction system.

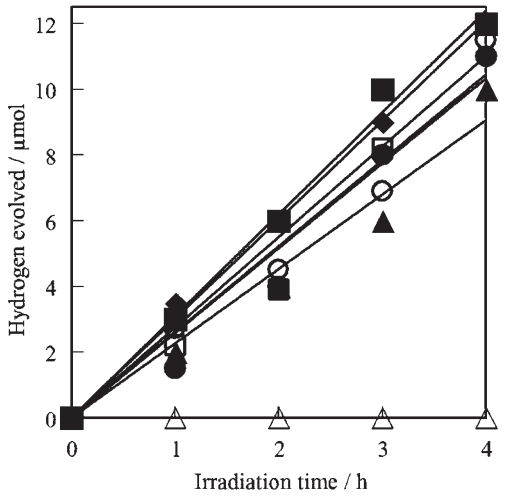

Figure 2. Time dependence of hydrogen production under steady state irradiation MC_n (0.3 mmol cellobiose unit), cellulase (4.0 units), NAD^+ (7.5 μmol), GDH (5.0 units), Mg chlorophyll-*a* (27 nmol), methylviologen (1.2 μmol) and platinum colloid (0.5 units) in 3 mL of 10 mmol dm^{-3} phosphate buffer (pH 7.0). Closed square: MC_1, closed circle: MC_2, closed triangle: MC_3, closed diamond: MC_4, open square: MC_5 ,open circle: MC_6 and open triangle: without irradiation.

CONCLUSIONS

Hydrogen production system coupling MC_n hydrolysis and hydrogen production with visible light-induced photosensitization of Mg chlorophyll-*a* was developed and continuous hydrogen gas production was achieved. Hydrogen production rate was not affected by the polymerization of MC_n. The cellulose derivative as a renewable bioresource is used effectively to produce environmentally clean, hydrogen as energy. Further research will bring about development of a hydrogen production system form cellulose contained in actual timber waste by photosensitization of Mg chlorophyll-*a*.

Acknowledgment. This work is partially supported by The Japan Securities Scholarship Foundation.

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