Photoinduced Hydrogen Production from Sucrose with the Combination System of Water-soluble Zinc Porphyrin and Enzymes

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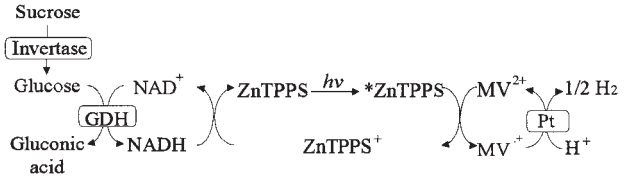
Photoinduced hydrogen production from sucrose as an electron-donating reagent has been investigated. When the sample solution containing sucrose, invertase, glucose dehydrogenase, nicotinamide adenine dinucreotide (NAD^+), zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS), methylviologen and colloidal platinum was irradiated, continuous hydrogen production was observed.

Hydrogen production system from the biomass resources is important in the environmental and the development of energy source research fields. Some renewable biomass resources are starch, cellulose, sucrose, lactose, and so on. These polysaccharides are hydrolyzed to form monosaccharides such as glucose. Thus, the conversion of glucose to hydrogen will be a useful new enzymatic pathway. Some studies on the hydrogen production from glucose using the enzymatic pathway have been reported.[1–4] The hydrogen production from glucose with the combination of the glucose dehydrogenase (GDH) and the hydrogenase has been reported.[5] However the enzymatic photoinduced hydrogen production from monosaccharides or polysaccharides such as sucrose has been paid little attention. Glucose was obtained from sucrose by using the invertase enzymatically. Thus, the hydrogen production from polysaccharide, sucrose will be attained using the combination of invertase, GDH and hydrogenase.

On the other hands, some photoinduced hydrogen production systems consisting of electron donor, photosensitizer, electron carrier and catalyst for hydrogen production are reported.[6,7] In these systems, zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and methylviologen are used as a photosensitizer and electron carrier, respectively. Colloidal platinum and hydrogenase form *Desulfovibrio vulgaris* (Miyazaki) are suitable catalyst for the hydrogen production. In the photoinduced hydrogen production system, photoexcited ZnTPPS reacts with methylviologen to form the reduced methylviologen and hydrogen evolves by the proton reduction with the catalyst and then the oxidized ZnTPPS is reduced by electron-donating reagent such as nicotinamide adenine dinucreotide (NADH). Thus, the electron donor, NADH was sacrificial reagent and the oxidized electron donor, NAD^+ was consumed in the reaction system. If the NADH is regenerated, the photoinduced hydrogen production system is accomplished without NAD^+ consumption. As GDH uses NAD^+ as a cofactor, the photoinduced hydrogen production system by the use of GDH and system consisting of electron donor, photosensitizer, electron carrier and catalyst will be attained.

In this work we describe the hydrogen production system coupling with the sucrose degradation by using the invertase, GDH and photoinduced hydrogen production with ZnTPPS and colloidal platinum as shown in Scheme 1.

Tetraphenylporphyrin tetrasulfonate (H_2TPPS) was purchased from Tokyo Chemical Industry Co. Ltd. Invertase from Yeast and glucose dehydrogenase (GDH) from *Bacillus sp.* were purchased from Wako Pure Chemical Industry Co. Ltd. NAD^+ and NADH were purchased from Oriental Yeast Co. Ltd. Zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) was synthesized by refluxing H_2TPPS with excess molar of zinc acetate in methanol and then was evaporated to dryness *in vacuo*.[8] Colloidal platinum was prepared by refluxing hydrogen hexachloplatinate(IV) hexahydrate and sodium citrate. The other chemicals were analytical grade or the highest grade available. The unit of GDH was defined as the reduction of 1.0 μmol NAD^+ to NADH by glucose per min.



Scheme 1. Enzymatic photoinduced hydrogen production system from sucrose.

The reaction was started by addition of NAD^+ (2.4 μmol) solution to the sample solution containing sucrose (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). The reduction of NAD^+ to NADH by GDH was determined using UV-vis spectrophotometer (Shimadzu Multispec-1500) at 340 nm. When the sample solution was incubated, the time dependence of the NADH formation was shown in Figure 1. After 80 min incubation, 1.0 μmol NADH was formed. Thus, the yield of the conversion of NAD^+ to NADH by the degradation of sucrose with invertase and GDH was *c.a.* 83%.

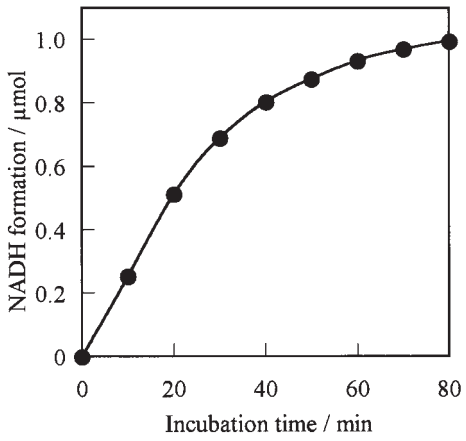


Figure 1. Time course of NADH formation form sucrose with the combination of invertase and GDH.

Next let us focus on the methylviologen photoreduction system containing NAD^+, sucrose, ZnTPPS, methylviologen, invertase and GDH. For the photoreaction under steady irradiation condition, 200 W tungsten lamp was used at 30 °C. The light of the wavelength less than 390 nm was cut by Toshiba L-39 cut-off filter. The reduction of methylviologen was determined using UV-vis spectrophotometer at 605 nm. When the deaerated sample solution containing NAD^+ (15 μmol), sucrose (0.30 mmol), ZnTPPS (3.0 nmol), methylviologen (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) was irradiated, the absorbance at 605 nm, absorption band of reduced methylviologen, increased with irradiation time. After 180 min irradiation, 1.1 μmol reduced methylviologen was produced. Thus, the conversion of methylviologen to the reduced methylviologen was *c.a.* 100% after 180 min irradiation.

As the methylviologen photoreduction system containing sucrose as an electron-donating reagent was achieved, the development of photoinduced hydrogen production system was attempted. The photoinduced hydrogen production from sucrose was carried out as follows. The sample solution containing sucrose (0.30 mmol), ZnTPPS (3.0 nmol), methylviologen (1.2 μmol), colloidal platinum (0.12 μmol), invertase (4.0 units) and GDH (5.0 units) in 10 mmol dm^{-3} phosphate buffer (pH=7.0) was deaerated using freeze pump thaw cycle for 6 times, and substituted by argon gas. The NAD^+ (15 μmol) solution, which was deaerated and substituted by argon gas, was added to the above solution and then the reaction was started by the irradiation. The reaction volume was 3.0 ml. The amount of hydrogen evolved was detected by gas chromatography (detector: TCD, column: active carbon, carrier gas: nitrogen). When the sample solution was irradiated, the hydrogen production was observed as shown in Figure 2. By irradiation, hydrogen evolving continued for more than 420 min. The amount of hydrogen production was estimated to be *c.a.* 18 μmol after 420 min irradiation. The amount of hydrogen evolved (18 μmol) was larger than that of the initial NAD^+ (15 μmol). This result indicates that NADH formed by invertase and GDH was oxidized to NAD^+ in the photoinduced hydrogen production and the formed NAD^+ was reduced to NADH by the degradation of sucrose by invertase and GDH, and then the formed NADH was used as an electron donor in the photoinduced hydrogen production.

The renewable biomass resources are used effectively to convert environmentally clean energy source, hydrogen gas. In the present system, the fructose consumption occurred because of the formation of glucose and fructose from sucrose by invertase. The system containing fructose dehydrogenation is being studied in detail.

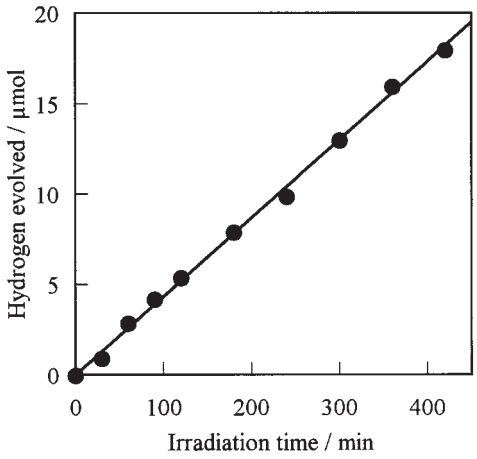


Figure 2. Photoinduced hydrogen production from sucrose using invertase, GDH, ZnTPPS and colloidal platinum.

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