Hydrogen Evolution from Glucose with the Combination of Glucose Dehydrogenase and Hydrogenase from *A. eutrophus* H16

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Hydrogen production system from glucose consisting of glucose dehydrogenase from *Bacillus sp.* and hydrogenase from *Alcaligenes eutrophus* H16 was established. When the solution containing glucose, glucose dehydrogenase, NAD, and hydrogenase was incubated at 30 °C, hydrogen evolution was observed.

Some renewable resources such as cellulose, starch, and lactose are contained in wastewater. Hydrogen production from the wastewater is of very importance as an environmentally clean energy source. As the above polysaccharides can easily be hydrolyzed to form glucose, the conversion of glucose to hydrogen will be a useful new enzymatic pathway.

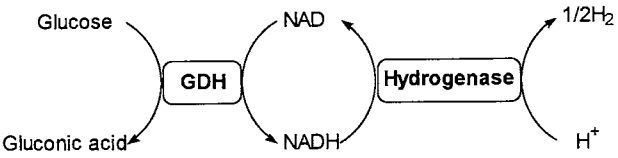
Though the hydrogen evolution system consisting of glucose dehydrogenase, NADP and the hydrogenase from *Thermoplasma acidophilum* and *Pyrococcus furiosus*[1-2] has been reported, the reaction system is undesirable for practical use as the optimum temperature of the reaction is pretty high for the enzyme is purified from thermophilic bacterium. A reaction system with mild conditions is desired.

The soluble hydrogenase of *Alcaligenes eutrophus* H16, mesophilic hydrogen oxidizing bacterium, catalyzes the hydrogen evolution from NADH, and has maximum activity at room temperature.[3] The reaction system with mild conditions can be achieved by the use of the hydrogenase from *A. eutrophus*. As the cofactor of this hydrogenase is NAD, many types of dehydrogenases can be used in the hydrogen evolution system, for these enzymes use NAD as a cofactor. This indicates the hydrogen can be evolved by the use of many types of dehydrogenase and the hydrogenase. In this study, the hydrogen production by the combination of glucose dehydrogenase and the soluble hydrogenase from *A. eutrophus* was carried out.

The soluble hydrogenase was partially purified from *A. eutrophus* according to the literature.[4] The unit of hydrogenase was defined as the reduction of 1 μmol NAD by H_2 per min. Glucose dehydrogenase from *Bacillus sp.* was purchased from Wako Pure Chemical Industry Co. The unit of glucose dehydrogenase was defined as the reduction of 1 μmol NAD by glucose per min. NAD and NADH were purchased from Oriental Yeast Co. and were purified before use.

The production of hydrogen from glucose was carried out as follows. The reaction mixture (4.1 cm^3) containing glucose (2.1 mmol), glucose dehydrogenase (10 units), and hydrogenase (4.5 units) in 5 cm^3 test tube was sealed with Septa and was deaerated by freeze pump thaw cycle for 3 times, and by flush with N_2. The reaction was started by adding N_2 saturated 4.1 μmol NAD solution to the reaction mixture. Produced hydrogen was measured by Shimadzu gas chromatograph with N_2 carrier. The reduction of NAD by glucose dehydrogenase was measured by UV spectrometer (Hitachi U-2000) at 340 nm.

When the solution containing hydrogenase (2.2 units) and NADH (1.2 mmol) was incubated at 30 °C under N_2 at pH 7.0, 70 μmol hydrogen was evolved after 40 min incubation confirming the hydrogen production from NADH with the hydrogenase from *A. eutrophus*. The hydrogenase found to catalyze the hydrogen evolution from NADH stoichiometrically, indicating that no side reaction of NADH oxidation occurred. When the solution containing glucose (2.1 mmol), glucose dehydrogenase (10 units), NAD (4.1 μmol), and hydrogenase (4.5 units) was incubated at 30 °C (pH 7.0), about 3 μmol of hydrogen was evolved after 1 h incubation. This demonstrates



Scheme 1. The hydrogen evolution system containing glucose dehydrogenase and hydrogenase. GDH: glucose dehydrogenase.

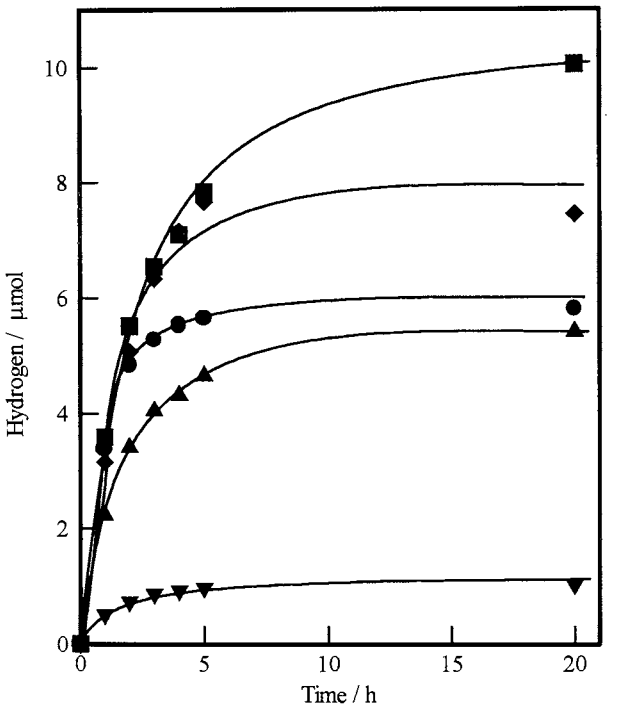


Figure 1. Time dependence of hydrogen evolution from glucose using hydrogenase from *A. eutrophus* and glucose dehydrogenase with different pH. ●: pH 6.0, ■: pH 6.5, ◆: pH 7.0, ▲: pH 7.5, ▼: pH 8.0.

the hydrogen evolution system from glucose consisting of glucose dehydrogenase, NAD, and hydrogenase as shown in Scheme 1.

Figure 1 shows time dependence of hydrogen evolution from glucose with different pH. The solution containing glucose, glucose dehydrogenase, NAD, and hydrogenase was incubated at 30 °C in different pH. In the pH range 7.0-8.0, 50 mM (1 M = 1 mol dm^{-3}) Tris-HCl buffer was used and 50 mM MES-KOH buffer was used in the pH range 6.0-6.5. The optimum pH for hydrogen evolution system from glucose was found to be pH 6.5. When the enzymatic system containing different types of enzymes, the optimum pH for each enzyme is sometimes different. The optimum pH for *A. eutrophus* hydrogenase was 6.5 to 7.0, and the optimum pH for the reduction of NAD by glucose dehydrogenase was found to be 8.0 at 30 °C. In this system, the reduction of NAD to NADH proceeds with the oxidation of glucose to gluconic acid and formed NADH is re-oxidized by the hydrogenase and hydrogen evolves. As the amount of hydrogen increased by additional hydrogenase, the rate-limiting step of this system is the hydrogen evolution from NADH by hydrogenase. The UV-visible absorption spectrum after 5 h incubation showed that the existence of NADH, supporting the rate-determining step of hydrogen evolution. Thus the optimum pH of the system is close to the optimum reaction condition for the hydrogenase.

As shown in Figure 1, hydrogen evolution stopped at every pH. By the addition of another hydrogenase after 21 h incubation, hydrogen evolution was observed again, showing the inactivation of hydrogenase in this reaction condition. To prolonged hydrogen evolution, the study is in progress.

The hydrogen evolution system containing glucose dehydrogenase and hydrogenase was established as shown in Scheme 1. In this system, the reduction of NAD to NADH proceeds with the oxidation of glucose to gluconic acid and formed NADH is re-oxidized by the hydrogenase and hydrogen evolves.

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References and Notes

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