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LETTER

Splitting of water by irradiation of visible light with a grana–NADP–FNR–NAD–hydrogenase system

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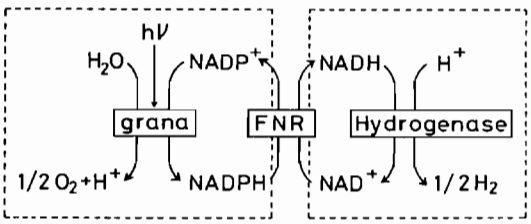
For photochemical utilization of solar energy, the splitting of water is one of the most important reactions to get hydrogen gas. Though the photochemical hydrogen evolution or oxygen evolution in the presence of sacrificial electron donors or acceptors has been studied extensively [1, 2], only a few systems of complete water splitting have been reported [3]. In this study, photochemical water splitting was tried by the combination of the following half reactions systems.

1. Oxygen evolution system by the irradiation of the grana from green plants in the presence of NADP as shown in the left-hand reaction of Scheme 1.



Scheme 1.

2. Hydrogen evolution system from NADH in the presence of hydrogenase from *Alcaligenes eutrophus*, which is specific for NADH, as shown in the right-hand reaction of Scheme 1.

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To combine the above two half reactions, ferredoxin NADP reductase (FNR) was used, as it can catalyze the following reaction

$$NAD^+ + NADPH \overset{FNR}{\rightleftharpoons} NADH + NADP^+$$

The grana from spinach leaves were prepared according to the literature [4]. The hydrogenase from *A. eutrophus* was partly purified according to the literature [5]. The unit of activity was the amount of hydrogenase used to reduce 1 μmol of NAD^+ for 1 min in the system containing hydrogenase and NAD^+ (2.0×10^{-4} mol dm^{-3}) in 4.0 ml of a 5.0×10^{-3} mol dm^{-3} Tris–HCl buffer (pH 8.0) under 300 torr hydrogen pressure at 30 °C.

Photoinduced water splitting was attempted by the combination of grana, FNR and hydrogenase. A reaction mixture containing grana, $NADP^+$, FNR, NAD^+ and hydrogenase in phosphate buffer (pH 8.0) was deaerated by repeated freeze–pump–thaw cycles. Then the reaction was started by irradiation with visible light from a 200 W tungsten lamp at 24 °C. Light of wavelength less than 550 nm was removed by a Kenko MC R-1 filter. Evolved hydrogen was analyzed by gas chromatography (Yanaco Co., Ld. G-1800). When the above system was irradiated hydrogen evolution was observed, and the amount of hydrogen increased with reaction time as shown in Fig. 1. When any component was omitted from the above system, no hydrogen evolution was observed. The amount of hydrogen evolved after 12 h irradiation was 0.24 μmol. By using the combination

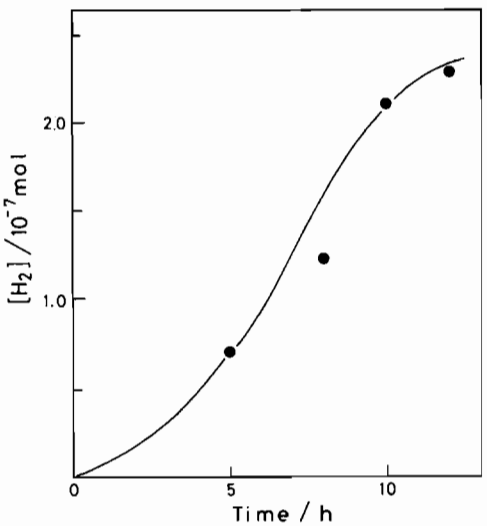

Fig. 1. Time dependence of hydrogen evolution. The sample solution (20 ml) contains $NADP^+$ (5.7×10^{-6} mol), NAD^+ (3.0×10^{-5} mol), grana (2 ml) containing FNR, and hydrogenase (100 μl) in 5.0×10^{-3} mol dm^{-3} phosphate buffer (pH 8.0). The reaction was carried out under anaerobic conditions with irradiation of visible light at 24 °C.

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grana–NADP–FNR–NAD–hydrogenase, photoinduced water splitting was accomplished.

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