Bull. Chem. Soc. Jpn. Vol. 82, No. 1, 93–95 (2009)

Photoinduced Hydrogen Production with a Platinum Nanoparticle and Light-Harvesting Chlorophyll *a*/*b*–Protein Complex of Photosystem II (LHCII) from *Spinach* System

Shuichi Ishigure,[1] Ayumi Okuda,[1] Kaoru Fujii,[1] Yuko Maki,[2] Mamoru Nango,*[1] and Yutaka Amao*[2]

[1]Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso, Nagoya 466-8555

[2]Department of Applied Chemistry, Oita University, 700 Dannoharu, Oita 870-1192

Received August 19, 2008; E-mail: amao@cc.oita-u.ac.jp

Photoinduced hydrogen production via the photoreduction of methyl viologen (MV^{2+}) using the sensitization of light-harvesting chlorophyll *a*/*b*–protein complex of photosystem II (LHCII) from *spinach* in the presence of platinum nanoparticles and NADH is developed.

Photoinduced hydrogen production from water, which is an attractive photosynthesis mimetic system, has been studied extensively by means of converting solar energy to chemical energy.[1–4] Photoinduced hydrogen production systems containing an electron-donor (D), a photosensitizer (P), an electron relay (C), and a hydrogen production catalyst have been widely studied. In this system, the photosensitizer molecule is an important factor. Water-soluble zinc porphyrins such as zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) and chlorophylls from green plants are used as photosensitizer molecules.[1–4] Recently, photosynthetic protein such as light-harvesting chlorophyll–protein complex (LHC), reaction center (RC), and photosystems I (PSI) and II (PSII) have been purified from green plants and photosynthetic bacteria using molecular biotechnology techniques. Especially photon-to-current conversion systems and photoinduced hydrogen production from water using the photosensitized functions of RC, PSI, and PSII have been attempted, because these proteins perform the important role of photoinduced charge separation. Although light-harvesting complex proteins (LHCs) perform the role of light harvesting and photoenergy transfer to reaction centers, they have not been paid much attention for these light energy conversion systems. However, chlorophylls and carotenoids are dye-molecules contained in LHCs. Carotenoids absorb blue and green region light, which is small molar coefficient of chlorophylls. Carotenoids serve two key roles in plants; one is absorption of light energy for use in photosynthesis, and the other is protection of chlorophyll from photodamage. Thus, LHCs are attractive photosensitizer material with wide absorption bands. Among the LHCs, light-harvesting chlorophyll-*a*/*b*–protein complex of photosystem II (LHCII), which plays the role of photoenergy transfer to PSII, purified from green plants such as *spinach* is stable compared with other photosynthetic proteins. LHCII consists of eight chlorophyll-*a*, six chlorophyll-*b*, two lutein, one 9′-*cis*-neoxanthin, and one violaxanthin, and in higher green plants is a major light-harvesting complex and serves as the principal solar energy collector in the photosynthesis of green plants and presumably also functions in photoprotection under high-light conditions.[5] As carotenoids such as lutein, 9′-*cis*-neoxanthin, and violaxanthin, and chlorophyll molecules are contained in LHCII, it is attractive photosensitizer material with wide absorption bands for photoenergy conversion systems.

In this article, we describe a system of methyl viologen (MV^{2+}) photoreduction by the visible light photosensitization of LHCII from *spinach* in the presence of NADH as an electron-donor.

LHCII was purified from *spinach* according to the literature.[6,7]

Photoreduction of MV^{2+} was carried out by the following method. A solution containing LHCII (chlorophyll concentration: 5.0 μmol dm^{-3}; the ratio of chlorophyll-*a*/*b* = 4/3), MV^{2+} (2.0 mmol dm^{-3}), and NADH (2.0 mmol dm^{-3}) in 3.0 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH 7) was deaerated by freeze–pump–thaw cycles repeated 6 times. LHCII was dissolved in aqueous solution with D-β-octylglucoside (OG) and 0.1 mmol dm^{-3} micellar hexadecyltrimethylammonium bromide (CTAB). The sample solution was irradiated with a 200-W halogen lamp (Philips) at a distance of 3.0 cm with a red-filter Sigma sharp-cut filter SCF-50S-60R (transmitted above the wavelength of 600 nm) or Sigma green filter GRF-50S-533G (transmitted between 450 and 550 nm light) at 30 °C. The solution containing chlorophyll *a*/*b* (total chlorophyll concentration: 5.0 μmol dm^{-3}; the ratio of chlorophyll-*a*/*b* = 4/3) instead of LHCII, MV^{2+} (2.0 mmol dm^{-3}), and NADH (2.0 mmol dm^{-3}) in 3.0 mL of 10 mmol dm^{-3} potassium phosphate buffer (pH 7) was used as a reference. Chlorophylls also were dissolved in aqueous solution with OG and 0.1 mmol dm^{-3} micellar CTAB. The MV^{2+} reduced form ($MV^{\bullet+}$) concentration was determined by the absorbance at 605 nm using the molar coefficient 13000 mol^{-1} dm^3 cm^{-1}.[8] When the sample solution was irradiated with a 200-W halogen lamp at a distance of 3.0 cm with a red-filter, corresponding to the absorption band of chlorophylls, the accumulation of $MV^{\bullet+}$ was observed with irradiation time as shown in Figure 1. For the system using LHCII (closed circles), the concentration of $MV^{\bullet+}$ was 0.18 mmol dm^{-3} and the conversion ratio of MV^{2+} to $MV^{\bullet+}$ was estimated to be 9.0% after 20 min irradiation. For the system using chlorophyll *a*/*b* (open circles) in contrast, the concentration of $MV^{\bullet+}$ was 0.14 mmol dm^{-3} and the conversion ratio was estimated to be 7.0%. In both systems, the concentration of chlorophyll *a*/*b* was almost the same. However, the MV^{2+} photoreduction was slightly increased using LHCII compared with the system using chlorophyll *a*/*b*.

When the sample solution was irradiated with a 200-W halogen lamp at a distance of 3.0 cm with a green-filter, corresponding to the absorption band of carotenoids, the

Published on the web January 14, 2009; doi:10.1246/bcsj.82.93

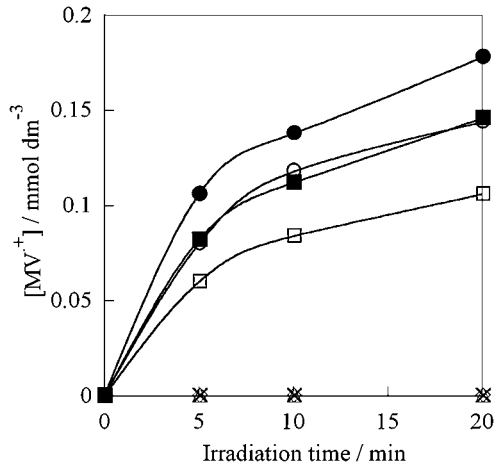

Figure 1. Time dependence of $MV^{\bullet+}$ production with the system consisting of NADH, LHCII or chlorophyll *a*/*b*, and MV^{2+} in potassium phosphate buffer (pH 7.0) under steady state irradiation at 30 °C. Circle: LHCII, square: chlorophyll *a*/*b*. Close: irradiation with the wavelength above 600 nm; open: irradiation with the wavelength between 400 and 500 nm. ×: Lutein used as a photosensitizer. Triangle: dark.

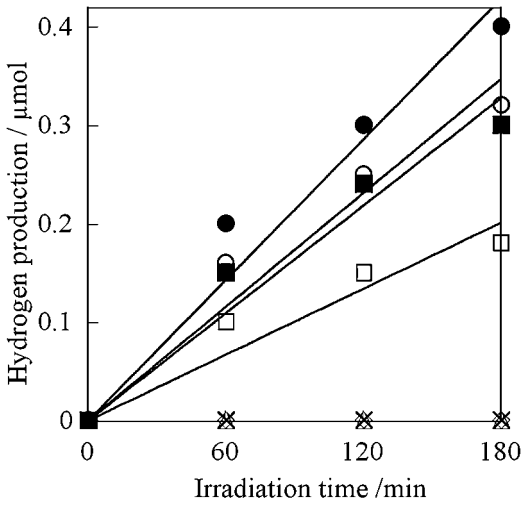

Figure 2. Time dependence of hydrogen production with the system consisting of NADH, LHCII or chlorophyll *a*/*b*, and MV^{2+} in potassium phosphate buffer (pH 7.0) under steady state irradiation at 30 °C. Circle: LHCII, square: chlorophyll *a*/*b*. Close: irradiation with the wavelength above 600 nm; open: irradiation with the wavelength between 400 and 500 nm. ×: Lutein used as a photosensitizer. Triangle: dark.

accumulation of $MV^{\bullet+}$ was observed with irradiation time as shown in Figure 1. For the system using LHCII (closed squares), the concentration of $MV^{\bullet+}$ was 0.15 mmol dm^{-3} and the conversion ratio of MV^{2+} to $MV^{\bullet+}$ was estimated to be 7.5% after 20 min irradiation. For the system using chlorophyll *a*/*b* (open squares) in contrast, the concentration of $MV^{\bullet+}$ was 0.09 mmol dm^{-3} and the conversion ratio was estimated to be 3.3%. The MV^{2+} photoreduction also was slightly increased using LHCII compared with the system using chlorophyll *a*/*b* with green light irradiation. In the system using LHCII, the conversion ratio of MV^{2+} to $MV^{\bullet+}$ was larger than that in the system using chlorophyll *a*/*b* with irradiation of the wavelength above 600 nm light. Lutein, which is carotenoid, exists in LHCII as a light-harvesting antenna dye molecule. Lutein has a strong absorption band between 400 and 500 nm. In Figure 1, the result of accumulation of $MV^{\bullet+}$ using lutein as a photosensitizer also is indicated (×). This result shows that the lower photoreduction activity of MV^{2+} is due to the shorter lifetime of the photoexicited state of lutein. Moreover, no photoreduction of MV^{2+} is observed without irradiation (open triangles). Therefore, effective MV^{2+} photoreduction also was developed using LHCII under green light irradiation.

As the MV^{2+} photoreduction system using LHCII was achieved, the development of a photoinduced hydrogen production system was attempted. The photoinduced hydrogen production with LHCII was carried out as follows. A sample solution containing LHCII (chlorophyll concentration: 5.0 μmol dm^{-3}; the ratio of chlorophyll-$a/b = 4/3$), MV^{2+} (2.0 mmol dm^{-3}), NADH (2.0 mmol dm^{-3}), and platinum nanoparticles (1.0 nmol) in 3.0 mL of 10 mmol dm^{-3} phosphate buffer (pH 7.0) was deaerated by freeze–pump–thaw cycle 6 times, and substituted by argon gas. The amount of hydrogen evolved was detected by gas chromatography (detector: TCD, column: active carbon, carrier gas: nitrogen). When the sample solution was irradiated with a 200-W halogen lamp at a distance of 3.0 cm with a red-filter, corresponding to the absorption band of chlorophylls, hydrogen production was observed with irradiation time as shown in Figure 2. For the system using LHCII (closed circles), the amount of hydrogen gas production was 0.40 μmol after 180 min irradiation. For the system using chlorophyll *a*/*b* (open circles) in contrast, the amount of hydrogen gas production was 0.29 μmol. In both systems, the concentration of chlorophyll *a*/*b* was almost the same. However, the amount of hydrogen production was slightly increased using LHCII compared with the system using chlorophyll *a*/*b*.

When the sample solution was irradiated with a 200-W halogen lamp at a distance of 3.0 cm with a green-filter, corresponding to the absorption band of lutein, the hydrogen production was observed with irradiation time as shown in Figure 2. For the system using LHCII (closed squares), the amount of hydrogen gas production was 0.28 μmol after 180 min irradiation. For the system using chlorophyll *a*/*b* (closed circles) in contrast, the amount of hydrogen gas production was 0.17 μmol. The photoinduced hydrogen production also was increased using LHCII compared with the system using chlorophyll *a*/*b* with green light irradiation. In the system using LHCII, the hydrogen production rate was larger than that in the system using chlorophyll *a*/*b* with irradiation at a wavelength above 600 nm. In Figure 2, the result of hydrogen production using lutein as a photosensitizer also is shown (×). Moreover, no hydrogen production was observed without irradiation (open triangles). Therefore, effective hydrogen production also was developed using LHCII under green light irradiation. When LHCII was excited with 480 nm corresponding to the absorption band of lutein, the fluorescence was observed around 700 nm to chlorophyll. It is predicted that the improvement in hydrogen production is due to the photoinduced energy transfer from lutein to chlorophyll in LHCII.

These results indicate that LHCII acts as an effective photosensitizer material with a wide absorption band for photoenergy conversion systems.

This work was partially supported by Hosokawa Powder Technology Foundation and Yazaki Science and Technology Foundation.

References

1 J. R. Darwent, P. Douglas, A. Harriman, G. Porter, M.-C. Richoux, *Coord. Chem. Rev.* **1982**, *44*, 83.

2 I. Okura, *Coord. Chem. Rev.* **1985**, *68*, 53.

3 Y. Amao, I. Okura, *Photocatalysis-Science and Technology*, KODANSHA-Springer, **2002**.

4 Y. Tomonou, Y. Amao, *BioMetals* **2003**, *16*, 419.

5 Z. Liu, H. Yan, K. Wang, T. Kuang, J. Zhang, L. Gui, X. An, W. Chang, *Nature* **2004**, *428*, 287.

6 J. J. Burke, C. L. Ditto, C. J. Arntzen, *Arch. Biochem. Biophys.* **1978**, *187*, 252.

7 Z. Krupa, H. P. A. Huner, J. P. Williams, E. Maissan, D. R. James, *Plant Physiol.* **1987**, *84*, 19.

8 T. Watanabe, K. Honda, *J. Phys. Chem.* **1982**, *86*, 2617.