Photoinduced Hydrogen Evolution with Hydrogenase and Cytochrome c_3

Toshiaki Kamachi, Tomohiro Hiraishi, and Ichiro Okura*
Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 227

(Received September 30, 1994)

The photoreduction of cytochrome c_3 using ZnTPPS as photosensitizer was investigated and the photoinduced hydrogen evolution with hydrogenase using cytochrome c_3 as electron carrier was established. The quenching of photoexcited triplet state of ZnTPPS with cytochrome c_3 was measured and the rate constant was 1.2 x 10^9 $M^{-1}s^{-1}$(1M=1 mol dm^{-3}).

Hydrogenases are the enzymes that catalyze the reversible oxidation of molecular hydrogen.[1] The photoinduced hydrogen evolution from water was studied extensively with the system composed of four components, that is, electron donating agent, photosensitizer, electron carrier and catalyst.[2] As we reported previously, the enzyme hydrogenase has been found to be a favorable catalyst for hydrogen evolution.[3] Zinc(II) tetraphenylporphyrin-4-sulfonate(ZnTPPS) is an active photosensitizer and methylviologen is a useful electron carrier. As the native electron carrier of the hydrogenase from *Desulfovibrio vulgaris* is cytochrome c_3[4], cytochrome c_3 may be better electron carrier than methylviologen for photoinduced hydrogen evolution. In this letter, we describe the photoreduction of cytochrome c_3 and photoinduced hydrogen evolution catalyzed by the hydrogenase.

Desulfovibrio vulgaris (Miyazaki) was cultured according to the literature.[5] The hydrogenase was solubilized from membrane fraction of *D. vulgaris* with trypsin and was purified according to the literature.[6] Cytochrome c_3 was purified as follows. After ultracentrifugation of the sonicated *D. vulgaris* cells, the supernatant was put on to DEAE Sepharose fast flow column followed by S Sepharose fast flow column preequilibrated with 25 mmol dm^{-3} Tris-HCl, pH 7.4. The adsorbed cytochrome c_3 was eluted with linear gradient of NaCl concentration in Tris-HCl buffer. The fraction containing cytochrome c_3 was concentrated by ultrafiltration with YM-5 membrane (Amicon Corp.) followed by gel filtration with Sephacryl S-200 column. The purified cytochrome c_3 gave single band on native polyacrylamide gel electrophoresis. The electron transfer between photoexcited ZnTPPS and cytochrome c_3 was measured using laser flash photolysis. For the excitation the second harmonic generation light of Nd-YAG laser (wavelength 532 nm, pulse width 10 ns) was used, and xenon lump was used as a monitor lamp. The sample solution was deaerated *in vacuo* to eliminate oxygen.

When the solution containing ZnTPPS, cytochrome c_3 and triethanolamine was irradiated, the reduction rate of cytochrome c_3 was pretty low. In the presence of methylviologen, cytochrome c_3 was easily photoreduced as shown in Figure 1. By the irradiation, a typical absorption band of cytochrome c_3 at 410 nm decreased and the absorption bands at 419, 528, 552 nm increased, indicating that the cytochrome c_3 was reduced. During the experiment, no reduced form of methylviologen was detected. These data indicate methylviologen is photoreduced much easier than cytochrome c_3, and the photoreduced methylviologen reduces cytochrome c_3 very fast.

Photoinduced hydrogen evolution was carried out by the irradiation of the system containing triethanolamine, ZnTPPS, methylviologen, hydrogenase and cytochrome c_3. The results are shown in Figure 2. In the presence of cytochrome c_3 hydrogen evolution was measured, while hydrogen was not evolved in the absence of cytochrome c_3. The induction time found in the presence of cytochrome c_3 may be the duration of reductive activation of hydrogenase, since the purified hydrogenase is inactive oxidized form and the reduction of hydrogenase is needed to get the activity of hydrogen evolution. These data indicate that cytochrome c_3 is better electron carrier for hydrogenase than methylviologen, though methylviologen is photoreduced easier than cytochrome c_3.

To determine the quenching rate of photoexcited triplet state of ZnTPPS with cytochrome c_3, T-T absorption of ZnTPPS was measured by laser flash photolysis. The results are shown in Figure 3. In the absence of cytochrome c_3, the decay of the excited triplet state of ZnTPPS obeyed first-order kinetics (Figure 3a). The quenching rate constant of photoexcited triplet state of ZnTPPS with cytochrome c_3 was determined to be 1.2 x 10^9 $M^{-1}s^{-1}$ by Stern-Volmer plots, indicating that cytochrome c_3 can quench triplet state of ZnTPPS with high efficiency. Though the photoexcited triplet state of ZnTPPS is quenched by cytochrome c_3, the cytochrome c_3 is not reduced. This may be caused by back electron transfer from cytochrome c_3 to ZnTPPS occurs as represented in scheme 1, path (4) and/or the efficiency of charge separation is low (scheme 1, path (2)).

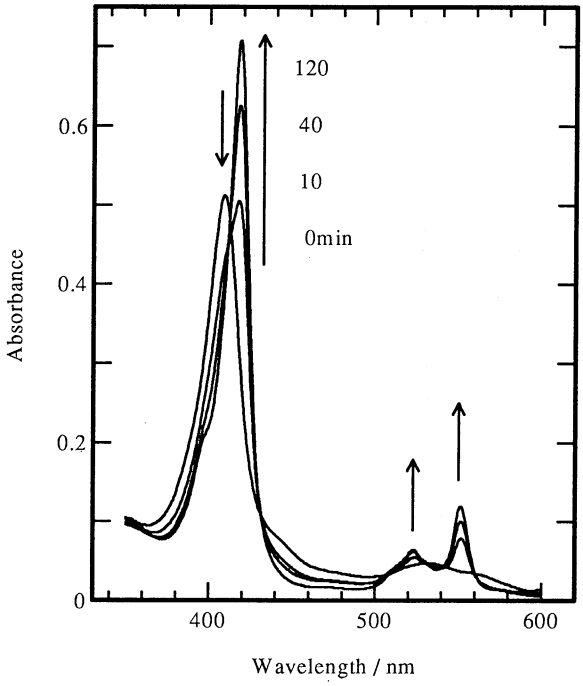


Figure 1. Change in absorption spectra of cytochrome c_3 during irradiation of W-lamp with an optical filter to cut less than 390nm at 30℃. The solution contains 1 nmol of triethanolamine, 0.3 nmol of ZnTPPS, 90 nmol of methylviologen and 5.7 nmol of cytochrome c_3 in 2.2 ml of 25 mmol dm^{-3} Tris-HCl buffer (pH 7.4).

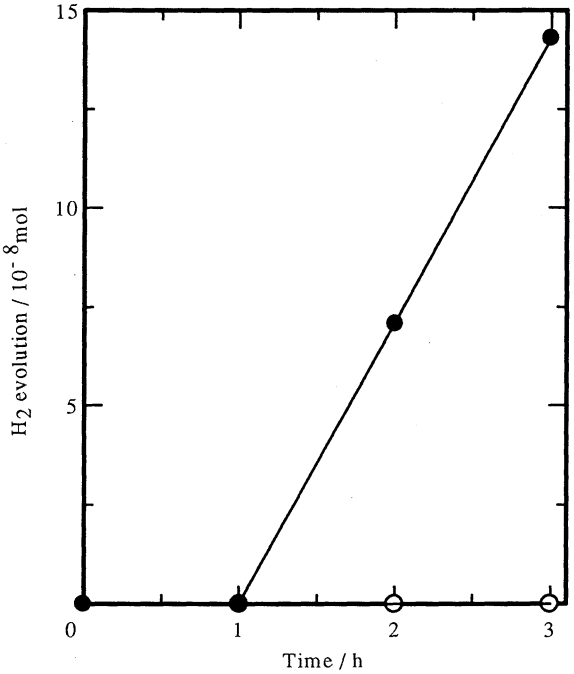


Figure 2. Time dependence of hydrogen evolution. The solution contained 1 mmol of triethanolamine, 0.5 nmol of ZnTPPS, 90 nmol of methylviologen, 0.3 unit of hydrogenase and in the absence of (-○-) or in the presence of (-●-) 6 nmol of cytochrome c_3 in 3.0 ml of 25 mmol dm^{-3} Tris-HCl buffer (pH 7.4).

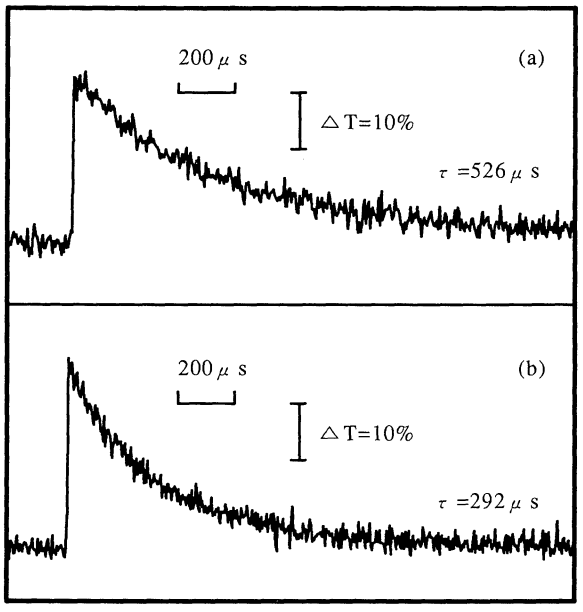
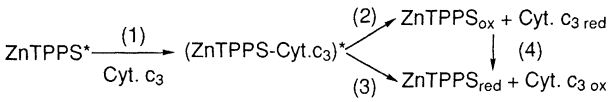
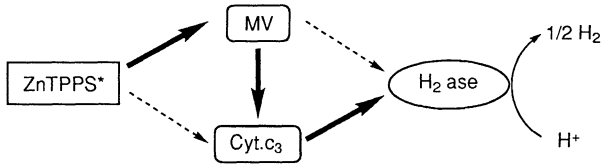


Figure 3. Decay of photoexcited triplet state of ZnTPPS (a) in the absence of or (b) in the presence of 1.2 μmol dm^{-3} of cytochrome c_3 in 2.2 ml of 25 mmol dm^{-3} Tris-HCl buffer (pH 7.4).



Scheme 1. Cyt. C_3: cytochrome c_3.

In the presence of methylviologen, cytochrome c_3 was easily reduced and photoinduced hydrogen evolution was detected only in the presence of cytochrome c_3, indicating that cytochrome c_3 is more efficient substrate of hydrogenase than methylviologen. The photoinduced hydrogen evolution will proceed by the scheme 2.



Scheme 2. MV:methylviologen, Cyt. c_3:cytochrome c_3, H_2ase: hydrogenase.

The photoexcited triplet state of ZnTPPS reacts easily with oxidized form of methylviologen than cytochrome c_3 so that reduced form of methylviologen generated. Then reduced methylviologen predominantly transfers the electron to cytochrome c_3. The reduced form of cytochrome c_3 reacts with hydrogenase and the hydrogen evolves.

This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Area "Organic Electrochemistry" (No. 06226226) from the Ministry of Education, Science and Culture, Japan.

References and Notes

1 M. W. W. Adams, *Biochim. Biophys. Acta*, **1020**, 115 (1990).
2 S. Aono and I. Okura, *J. Phys. Chem.*, **89**, 1593 (1985).
3 I. Okura, S. Kusunoki, N. Kim-Thuan and M. Kobayashi, *J. Chem. Soc., Chem. Commun.*, **1981**, 56.
4 M. W. W. Adams, L. E. Mortenson and J. S. Chen, *Biochim. Biophys. Acta*, **594**, 105 (1981).
5 T. Yagi, K. Kimura, H. Daidoji, F. Sakai, S. Tamura and H. Inokuchi, *J. Biochem.*, **79**, 241 (1976).
6 T. Yagi, K. Kimura, H. Daidoji, F. Sakai, S. Tamura and H. Inokuchi, *J. Biochem.*, **79**, 661 (1976).