# Photoinduced Hydrogen Evolution Using Cytochrome $c_3$ as a Mediator

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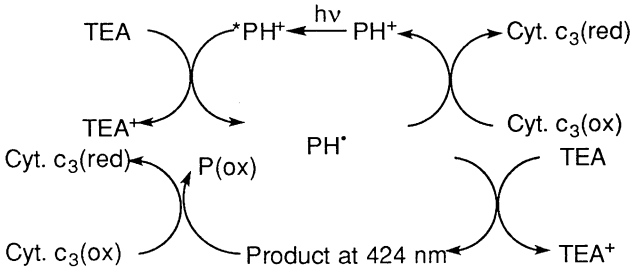
Photoreduction of cytochrome $c_3$ using proflavin was investigated and photoinduced hydrogen evolution with hydrogenase using cytochrome $c_3$ as an electron carrier was established. The photoexcited proflavin was quenched by triethanolamine, followed by cytochrome $c_3$ reduction, and hydrogen evolution catalyzed by hydrogenase.

The hydrogen production using solar energy has been studied exclusively as a means of solar energy conversion to chemical energy.[1] For photoinduced hydrogen evolution in homogeneous system, four components, an electron donor, a photosensitizer such as zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS), an electron carrier, and a catalyst such as hydrogenase have been widely used. As we reported previously, photoinduced hydrogen evolution was observed when cytochrome $c_3$, a natural electron carrier of hydrogenase, and methylviologen were used as electron carriers. In such system, one of the important processes is the charge separation between photoexcited ZnTPPS and cytochrome $c_3$. However, ZnTPPS was ineffective for photoreduction of cytochrome $c_3$ responsible for the back electron transfer between reduced cytochrome $c_3$ and ZnTPPS cation radical.[2] In this paper we searched for the more effective photosensitizer than ZnTPPS in this system. When proflavin, 3,6-diaminoacridine, was used as a photosensitizer instead of ZnTPPS, cytochrome $c_3$ was effectively photoreduced and efficient photoinduced hydrogen evolution was accomplished, and the process of cytochrome $c_3$ photoreduction with proflavin was discussed.

Hydrogenase and cytochrome $c_3$ from *Desulfovibrio vulgaris* (Miyazaki ) were purified according to the literature.[2,3] Protein concentrations were determined using the following molar absorption coefficients: for cytochrome $c_3$, $\varepsilon$=110 mmol$^{-1}$ dm$^3$ cm$^{-1}$ at the reduced $\alpha$-band maximum (552 nm): for hydrogenase, $\varepsilon$=47 mmol$^{-1}$ dm$^3$ cm$^{-1}$ at 400 nm. The sample solutions were prepared in 25 mmol dm$^{-3}$ Tris-HCl buffer (Tris, tris-(hydroxym ethyl)aminomethane) (pH 7.4) and were deaerated by repeated freeze-pump-thaw cycles. For the photolysis under steady state irradiation, 200 W tungsten lamp was used at 30 °C. The light of wavelength less than 390 nm was removed by Toshiba L-39 cut-off filter. Evolved hydrogen was detected by gas chromatography (detector: TCD, column: active carbon).

When a sample solution containing proflavin, protonated under experimental conditions (pH 7.4), cytochrome $c_3$ and triethanolamine (TEA) was irradiated, the spectrum of cytochrome $c_3$ changed as shown in Figure 1. A typical absorption band (at 410 nm) of the oxidized state of cytochrome $c_3$ decreased and typical absorption bands of reduced cytochrome $c_3$ at 419, 528 and 552 nm increased, indicating that cytochrome $c_3$ was reduced.

To determine the process of cytochrome $c_3$ photoreduction with proflavin, the photolysis of proflavin in the presence of TEA or cytochrome $c_3$ was investigated. When a solution containing proflavin and TEA was irradiated, the absorption band at 445 nm, attributed to proflavin cation, decreased and the absorption band at 424 nm appeared. The photoreduced product attributed to 424 nm, not characterized in detail, has also been reported with the system of proflavin and ethylenediaminetetraacetic acid.[4] On the other hand, when a solution containing proflavin and cytochrome $c_3$ was irradiated, no absorption change was observed. These results indicate cytochrome $c_3$ is reduced with proflavin radical or photoproduct at 424 nm as shown in Scheme 1. The main




**Scheme 1.** TEA: triethanoleamine, $PH^+$: proflavin cation Cyt. $c_3$: cytochrome $c_3$ , $PH^•$: proflavin radical P(ox): inactive form of proflavin .

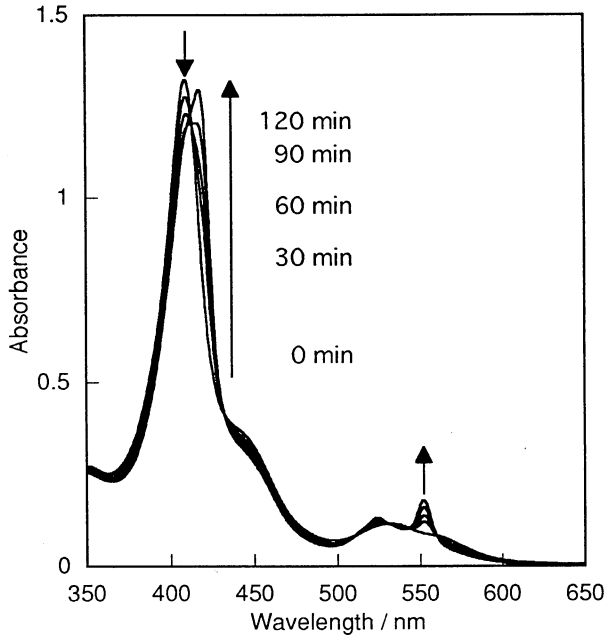



**Figure 1.** Absorption spectrum change of cytochrome $c_3$ during irradiation at 30 °C. The sample solution contains triethanolamine (0.33 mol dm$^{-3}$), proflavin (5.0 μmol dm$^{-3}$) and cytochrome $c_3$ (2.7 μmol dm$^{-3}$) in 2.0 ml of 25 mmol dm$^{-3}$ Tris-HCl buffer (pH 7.4).

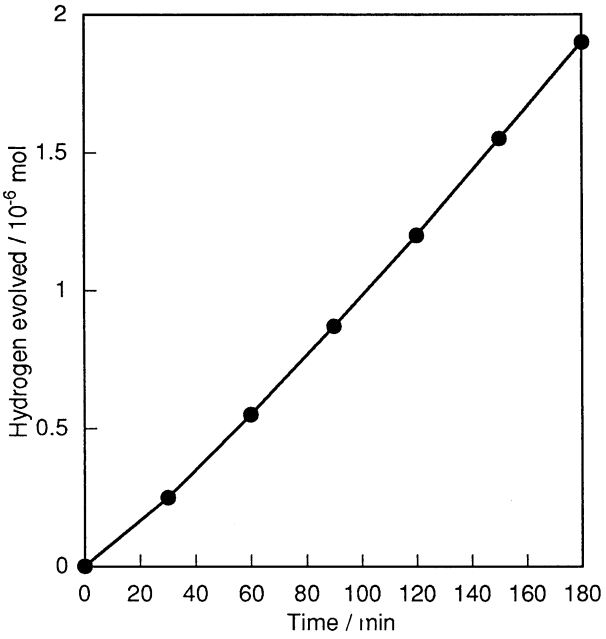



**Figure 2.** Time dependence of hydrogen evolution. The sample solution containing triethanolamine (0.33 mol $dm^{-3}$), proflavin (67 μmol $dm^{-3}$), cytochrome $c_3$ (1.6 μmol $dm^{-3}$) and hydrogenase (86 nmol $dm^{-3}$) in 3.0 ml of 25 mmol $dm^{-3}$ Tris - HCl buffer (pH 7.4) is irradiatedat 30 °C.

pathway is that photoexcited proflavin is quenched by TEA to form proflavin radical and then electron transfers from proflavin radical to cytochrome $c_3$. On the laser flash photolysis study, however, photoexcited proflavin cation was not observed, so that the reductive quenching rate was not determined. The second pathway is that photoproduct at 424 nm is formed by proflavin radical and TEA, so that electron transfers from photoproduct to cytochrome $c_3$ and proflavin becomes an inactive form.

As the effective photoreduction of cytochrome $c_3$ was accomplished using proflavin, photoinduced hydrogen evolution was carried out by the irradiation of the sample solution containing TEA, proflavin, cytochrome $c_3$ and hydrogenase. Figure 2 shows the time dependence of hydrogen evolution. In this system the steady photoinduced hydrogen evolution was observed and the amount of hydrogen evolved was 1.55 μmol for 150 min irradiation. The quantum yield of the photoinduced hydrogen evolution was 1.8%. On the other hand, when ZnTPPS was used as a photosensitizer, no photoinduced hydrogen evolution was observed for 180 min irradiation. From the above results proflavin is an useful photosensitizer for photoinduced hydrogen evolution using cytochrome $c_3$ as an electron carrier.

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## References and Notes

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