

PHOTOREDUCTION OF CYTOCHROME C_3 AND HYDROGEN EVOLUTION
WITH HYDROGENASE

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The formation of the reduced form of cytochrome c_3 was established when an aqueous solution containing Zn-TPPS₃, cytochrome c_3 and triethanolamine was irradiated by visible light. When hydrogenase was added to the system hydrogen evolution was observed by the irradiation of sunlight.

The enzyme hydrogenase can catalyze the decomposition of water in the presence of an electron donating agent which should be a substrate of the enzyme and which should also have an enough redox potential for the decomposition of water. The electron donating agent employed almost exclusively is the reduced form of methyl viologen (1,1'-dimethyl-4,4'-bipyridinium chloride). The reduction of methyl viologen with the aid of light energy has been studied extensively on the purpose of the conversion of solar energy into chemical energy.¹⁾ Cytochrome c_3 , however, is more suitable substrate of hydrogenase than methyl viologen^{2,3)}, and is expected to be an efficient electron carrier for photoinduced hydrogen evolution. We report here the photoreduction of cytochrome c_3 by the irradiation of the system containing photosensitizer, cytochrome c_3 and triethanolamine as a reducing agent, and hydrogen evolution catalyzed by hydrogenase.

Desulfovibrio vulgaris (Miyazaki type) cells were cultured according to the literature.⁴⁾ The hydrogenase²⁾ and cytochrome c_3 ⁵⁾ from *D. vulgaris* are purified by Yagi's methods. Zinc-meso-tetraphenylporphyrintrisulfonic acid (Zn-TPPS₃) was synthesized by refluxing equimolar TPPS₃ and zinc acetate in methanol for 30 min. The following procedure, under anaerobic conditions, is typical. To 3.51×10^{-9} mol of Zn-TPPS₃, 2.30×10^{-9} mol of cytochrome c_3 , and 1.0×10^{-3} mol of triethanolamine, 1.0 cm^3 of hydrogenase was added. The concentration of hydrogenase was not known, but 1.4×10^{-6} mol of hydrogen was generated by the following reaction system: hydrogenase (0.5 cm^3) - methyl viologen (2.10×10^{-6} mol) - $\text{Na}_2\text{S}_2\text{O}_4$ (2.30×10^{-5} mol) in 3.0 cm^3 of 0.02 M Tris-HCl buffer (pH 7.0) at 30°C for 10 min. The mixture was adjusted to 6.0 cm^3 with 0.02 M Tris-HCl buffer (pH 7.0, a value suitable for hydrogenase). This solution was then irradiated (150 W slide projector tungsten lamp) in a Pyrex reaction vessel at 30°C. Light with wavelength shorter than 390 nm was excluded by the use of Toshiba L-39 filter. A sample of the evolved hydrogen was collected with a sampling valve and analyzed by g.l.c.

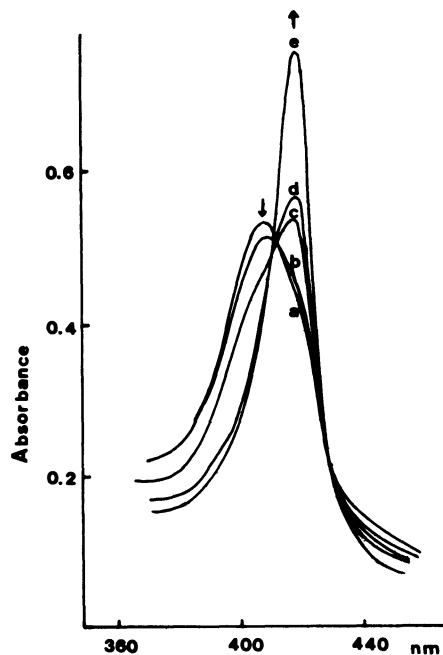


Figure Change of spectrum with irradiation time. The solution containing Zn-TPPS₃, cytochrome c₃ and triethanolamine was irradiated for 0 min(a), 6(b), 11(c), 52(d) and 117(e).

When an aqueous solution containing Zn-TPPS₃, cytochrome c₃, and triethanolamine was irradiated, the spectrum change of cytochrome c₃ was observed in the figure. The spectrum with 419 nm attributed to oxidized form of cytochrome c₃ decreased and the spectrum with 432 nm appeared. As the reduction of cytochrome c₃ with dithionite yielded the same spectrum, the chemical species with the spectrum with 432 nm can be attributed to reduced form of cytochrome c₃.⁵⁾ The concentration of the reduced form of cytochrome c₃ increased rapidly at the beginning of the reaction and reached a constant value. After 2 h irradiation almost all of cytochrome c₃ was reduced and existed in the reduced form. Aeration of the product leads to immediate reconversion to the starting compound, the oxidized form of cytochrome c₃, without loss.

When 2.80×10^{-8} mol of Ru(bpy)₃²⁺ or 2.36×10^{-8} mol of hematoporphyrin was used in place of Zn-TPPS₃, no reduction of cytochrome c₃ was observed, though the concentrations of Ru(bpy)₃²⁺ and hematoporphyrin were much higher than Zn-TPPS₃. The same tendency has been observed on the photoreduction of methyl viologen.⁶⁾

When hydrogenase (1.0 cm^3) was added to the system containing Zn-TPPS₃, cytochrome c₃ and triethanolamine and irradiated by sunlight for 6 h, hydrogen evolution (7.3×10^{-7} mol) was observed. No hydrogen evolution was observed when any component of the system was omitted.

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