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Effective photoinduced hydrogen evolution with hydrogenase in surfactant micelles

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Abstract

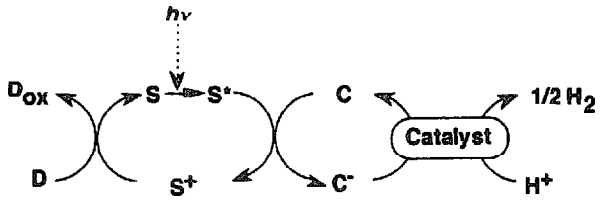
Photoinduced hydrogen evolution with hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) by the combination of three component system consisting of triethanolamine, zinc–tetraphenylporphyrin tetrasulfonate ($ZnTPPS_4$) and methyl viologen was investigated in cationic surfactant, cetyltrimethylammonium bromide (CTAB), anionic surfactant, sodium dodecyl sulfate (SDS) or non ionic surfactant, Triton X-100 micellar solution by steady state irradiation. The rate of photoreduction of methyl viologen increased by the addition of surfactants, especially CTAB. The back electron transfer reaction from reduced methyl viologen to $ZnTPPS_4$ cation radical was strongly suppressed by the addition of various surfactants, especially ionic surfactant CTAB or SDS. The effective hydrogen evolution from reduced methyl viologen with hydrogenase was accomplished in the presence of CTAB or Triton X-100 micelle.

Keywords: Hydrogenase; Photoinduced hydrogen evolution; CTAB; Triton X-100; SDS

1. Introduction

Photochemical redox reactions have been studied extensively by means of converting solar energy to chemical energy. Three component systems consisting of an electron donor (D), a photosensitizer (S) and an electron carrier (C) have been widely used to produce hydrogen from water in the presence of a suitable catalyst as shown in Scheme 1 [1,2].

In this reaction, the charge separation to form oxidized photosensitizer (S^+) and reduced electron carrier (C^-) is one of the important steps [3,4]. The effective charge separation has been attempted by using micellar system [5], colloidal inorganic suspension [6] or liposomes [7]. As



Scheme 1.

we reported previously the effective charge separation was accomplished in sodium dodecyl sulfate, SDS, micellar system [8,9]. However, little hydrogen evolution with hydrogenase was observed because of the denaturation of hydrogenase by SDS.

In this study, the effective charge separation between a photosensitizer and an electron carrier was accomplished in the presence of various surfactants, especially cationic surfactant and an

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effective hydrogen evolution with hydrogenase was observed in CTAB or Triton X-100 micelle.

2. Experimental

2.1. Materials

Zinc tetraphenylporphyrin tetrasulfonate ($ZnTPPS_4$) was synthesized by refluxing tetraphenylporphyrin tetrasulfonate (Dojin Laboratories) with about ten times molar equivalent of zinc acetate (Kanto Chemical Co., Inc.) in methanol for 2 h. After evaporation of the solvent in vacuo, the product was dissolved in distilled water. All the other chemicals were obtained from commercial sources and were used as received.

2.2. Purification of hydrogenase

Hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) was purified according to Yagi's method [10]. The hydrogenase concentration is not known, but it has the ability to release 0.7 μmol of hydrogen in the reaction system of 10 μl hydrogenase, 1.2×10^{-5} mol of methyl viologen and 7.7×10^{-5} mol sodium dithionite in 5.0 ml of 50 mmol dm^{-3} Tris–HCl buffer (pH = 7.4) at 30°C for 10 min.

2.3. Photoreduction of methyl viologen and hydrogen evolution by steady state irradiation

In photolysis under steady state irradiation, the sample solution in a Pyrex cell with magnetic stirrer was irradiated with 200 W tungsten lamp (Philips KP-8) at 30°C. Light of wavelengths less than 390 nm was removed by Toshiba L-39 filter. For the photoreduction of methyl viologen, the sample solution consisting of 0.25 mol dm^{-3} triethanolamine, 1.3×10^{-7} mol dm^{-3} $ZnTPPS_4$, 2.2×10^{-4} mol dm^{-3} methyl viologen and surfactant was deaerated by repeated freeze–pump–thaw cycles. The concentration of reduced methyl viologen was determined by visible spectra (U2000 Hitachi) at 605 nm ($\varepsilon = 1.1\times10^{4}$ cm^{-1} mol^{-1} dm^3). For hydrogen evolution with hydrogenase, the sample solution consisting of 0.25 mol dm^{-3} triethanolamine, 1.3×10^{-7} mol dm^{-3} $ZnTPPS_4$, 2.2×10^{-4} mol dm^{-3} methyl viologen, 50 μl of hydrogenase and surfactant was deaerated and the evolved hydrogen was detected by gas chromatography (Shimadzu GC-14B, detector: TCD, column: active carbon).

2.4. Laser flash photolysis

Laser flash photolysis was carried out by using Nd-YAG laser (Spectra Physics Quanta Ray DCR-3) with second harmonic light with 532 nm (pulse width 10 ns) at room temperature. Xenon arc lamp was used as a monitoring light beam. The transient spectra were stored in storage oscilloscope (SONY-Tektronix 11401).

3. Results and discussion

3.1. Photoreduction of methyl viologen by steady state irradiation

When the sample solution containing triethanolamine, $ZnTPPS_4$, methyl viologen, and surfactant was irradiated, the accumulation of reduced methyl viologen was observed as shown in Fig. 1.

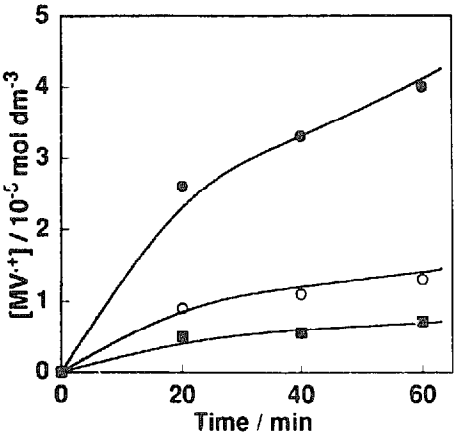

Fig. 1. Time dependence of reduced methyl viologen formation. The sample solution containing triethanolamine (0.25 mol dm^{-3}), $ZnTPPS_4$ (0.13 μmol dm^{-3}) and methyl viologen (0.22 mmol dm^{-3}) in 25 mmol dm^{-3} Tris–HCl buffer (pH = 7.4) was irradiated at 30°C. (■) without surfactant; (○) 40 mmol dm^{-3} Triton X-100; (●) 25 mmol dm^{-3} CTAB.

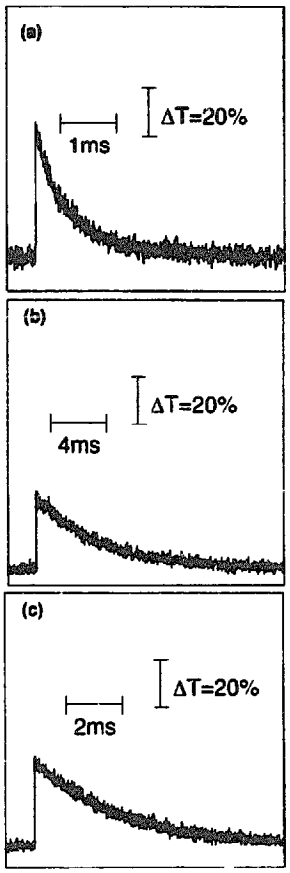

Fig. 2. Decay of photoexcited triplet state of $ZnTPPS_4$ after laser flash monitored at 470 nm. The sample solution contains $ZnTPPS_4$ (30 μmol dm^{-3}) and surfactant in 25 mmol dm^{-3} Tris–HCl buffer (pH = 7.4). (a) without surfactant; (b) 40 mmol dm^{-3} Triton X-100; (c) 25 mmol dm^{-3} CTAB.

Table 1
Lifetime of photoexcited triplet $ZnTPPS_4$ (τ) in various surfactants

Surfactant	τ/ms
None	0.60
CTAB (25 mmol dm^{-3})	3.0
Triton X-100 (40 mmol dm^{-3})	4.2
SDS (25 mmol dm^{-3})	0.61

The reduced methyl viologen concentration increased with irradiation time. The rate of photoreduction of methyl viologen increased by the addition of surfactants, especially remarkable increase was observed by the addition of CTAB. To clarify the reason of the rate increase of photoreduction of methyl viologen by the addition of surfactant, the elementary processes of photoreduction of methyl viologen was studied by using laser flash photolysis.

3.2. Lifetime of photoexcited triplet state of $ZnTPPS_4$

As the lifetime of photoexcited triplet state of $ZnTPPS_4$ plays an important role for photoreaction rate, the effect of surfactant on the lifetime of photoexcited triplet state of $ZnTPPS_4$ was measured by laser flash photolysis. Typical decay curves of photoexcited triplet state of $ZnTPPS_4$ in surfactant micelles observed at 470 nm after laser flash were shown in Fig. 2. Every decay was followed first order kinetics. The lifetime of the photoexcited triplet state of $ZnTPPS_4$ in various surfactants were listed in Table 1. In the presence of CTAB or Triton X-100, the lifetime increased dramatically. Effect of surfactant concentration on the lifetime of photoexcited triplet state of $ZnTPPS_4$ was shown in Fig. 3. When the surfactant, CTAB or Triton X-100 was added to the $ZnTPPS_4$ solution, the absorption spectra of $ZnTPPS_4$ changed with the isosbestic point. This result suggests that the complex was formed between $ZnTPPS_4$ and surfactant micelles. In the case of SDS, no complex may be formed due to the electrostatic repulsion between negatively charged $ZnTPPS_4$ and negative surface charge of SDS. The increase of lifetime may be induced by the com-

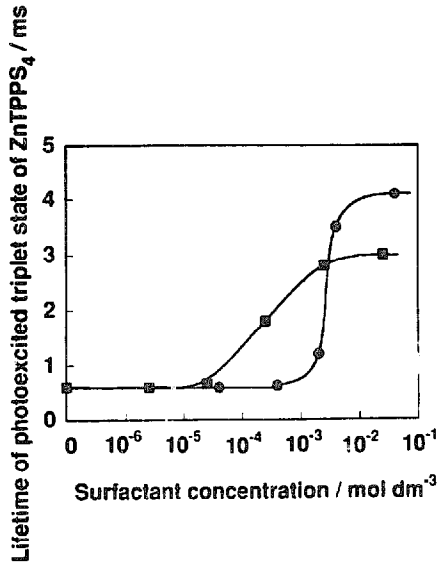

Fig. 3. Lifetime of photoexcited triplet state of $ZnTPPS_4$ vs. surfactant concentration. The sample solution contains $ZnTPPS_4$ (30 μmol dm^{-3}), and surfactant in 25 mmol dm^{-3} Tris–HCl buffer (pH = 7.4). (■) CTAB; (●) Triton X-100.

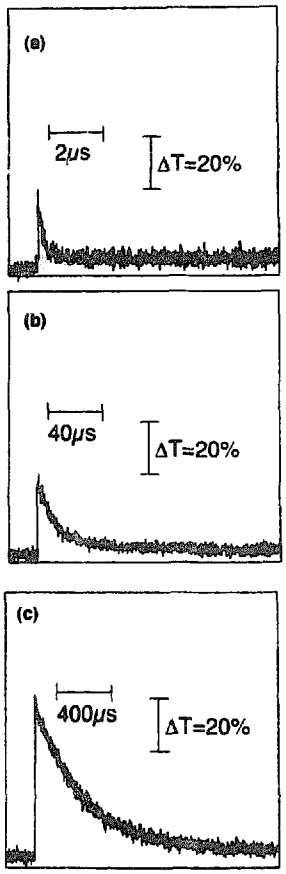

Fig. 4. Decay of photoexcited triplet state of $ZnTPPS_4$ in the presence of methyl viologen after laser flash monitored at 470 nm. (a): $ZnTPPS_4$ (30 μmol dm^{-3}) and methyl viologen (0.75 mmol dm^{-3}); (b): $ZnTPPS_4$ (30 μmol dm^{-3}), methyl viologen (0.75 mmol dm^{-3}) and 40 mmol dm^{-3} Triton X-100; (c): $ZnTPPS_4$ (30 μmol dm^{-3}), methyl viologen (0.75 mmol dm^{-3}) and 25 mmol dm^{-3} CTAB in 25 mmol dm^{-3} Tris–HCl buffer (pH = 7.4).

plex formation between the surfactant micelle such as CTAB or Triton X-100, and $ZnTPPS_4$.

3.3. Quenching of photoexcited triplet state of $ZnTPPS_4$ by methyl viologen

Typical decay curves of photoexcited triplet state of $ZnTPPS_4$ in the presence of methyl viologen in surfactant micelles observed at 470 nm after laser flash were shown in Fig. 4. From the lifetime of photoexcited triplet state of $ZnTPPS_4$, it was clarified that the quenching of photoexcited triplet state of $ZnTPPS_4$ by methyl viologen was suppressed by the addition of surfactant. The quenching rate constant, k_q, in the presence of 2.5×10^{-2} mol dm^{-3} CTAB was estimated to be 3.3×10^{6} mol^{-1} dm^3 s^{-1} by Stern–Volmer plot. Though the quenching rate was suppressed by the addition of surfactants, the quenching efficiency was not affected. The quenching efficiency of photoexcited triplet state of $ZnTPPS_4$ by methyl viologen, η_q, is expressed as follows.

$$\eta_q = k_q[MV^{2+}]/(k_0 + k_q[MV^{2+}])$$

where k_0 is the inverse of the lifetime of photoexcited triplet state of $ZnTPPS_4$ in the absence of methyl viologen. Table 2 shows k_q and η_q in the presence of various surfactants. By the addition of ionic surfactant, CTAB or SDS, suppression of the quenching rate may be caused by the electrostatic effect among the surface charge of surfactant, $ZnTPPS_4$ and methyl viologen. In the presence of Triton X-100, the quenching reaction rate was not suppressed.

3.4. Back electron transfer between reduced methyl viologen and $ZnTPPS_4$ cation radical

Typical decay curves of reduced methyl viologen in surfactant micelles observed at 605 nm after laser flash were shown in Fig. 5. Every decay followed second order kinetics. The lifetime of reduced methyl viologen in the presence of 2.5×10^{-2} mol dm^{-3} CTAB was pretty long, about 100 times as long as in the absence of surfactants. To clarify the micellar effect on the lifetime of reduced methyl viologen, the surfactant concentration dependence on lifetime of reduced methyl viologen monitored at 605 nm after laser flash was measured and the results are shown in Fig. 6. The lifetime of reduced methyl viologen increased with surfactant concentration. The back electron transfer rate constant from reduced

Table 2
Rate constants for quenching k_q and quenching efficiency, η_q in various surfactants

Surfactant	k_q/mol^{-1} dm^3 s^{-1}	η_q
None	1.7×10^{9}	0.99
CTAB	3.3×10^{6}	0.85
Triton X-100	1.0×10^{9}	0.99
SDS [a]	8.3×10^{7}	0.85

[a] Data from [8].

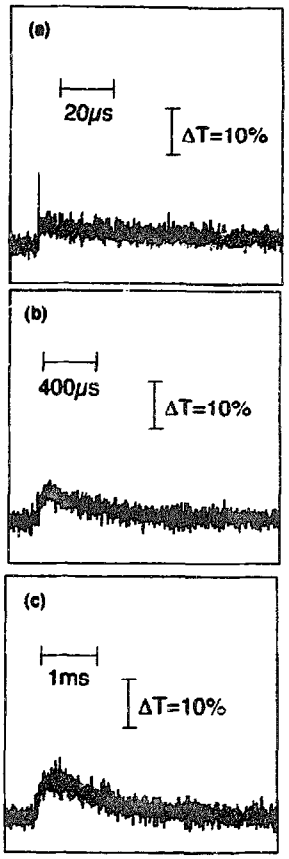

Fig. 5. Decay of reduced methyl viologen after laser flash monitored at 605 nm. (a): $ZnTPPS_4$ (30 μmol dm^{-3}) and methyl viologen (0.75 mmol dm^{-3}); (b): $ZnTPPS_4$ (30 μmol dm^{-3}), methyl viologen (0.75 mmol dm^{-3}) and 40 mmol dm^{-3} Triton X-100; (c): $ZnTPPS_4$ (30 μmol dm^{-3}), methyl viologen (0.75 mmol dm^{-3}) and 25 mmol dm^{-3} CTAB in 25 mmol dm^{-3} Tris–HCl buffer (pH = 7.4).

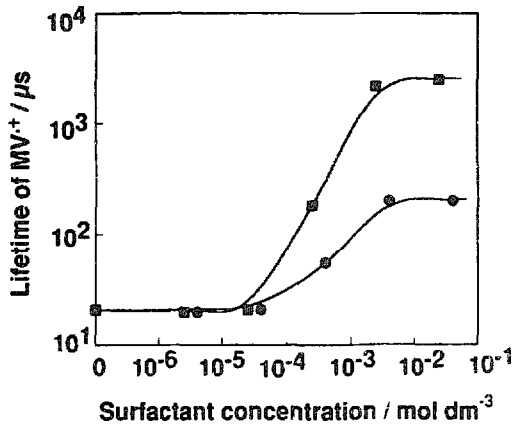

Fig. 6. Lifetime of reduced methyl viologen vs. surfactant concentration. (■) $ZnTPPS_4$ (30 μmol dm^{-3}), methyl viologen (0.75 mmol dm^{-3}) and CTAB; (●) $ZnTPPS_4$ (30 μmol dm^{-3}), methyl viologen (0.75 mmol dm^{-3}) and Triton X-100.

methyl viologen to $ZnTPPS_4$ radical cation in the presence of 2.5×10^{-2} mol dm^{-3} CTAB was estimated to be 5.7×10^7 mol^{-1} dm^3 s^{-1} as compared to 8.9×10^9 mol^{-1} dm^3 s^{-1} in absence of surfactants. The lifetime of reduced methyl viologen and the back electron transfer rate constant in various surfactants were listed in Table 3. For CTAB and SDS, the retardation of back electron transfer reaction is explained by an electrostatic effect among the surface charge of CTAB or SDS micelle, $ZnTPPS_4$, and methyl viologen. The charge recombination of the reduced methyl viologen and $ZnTPPS_4$ cation radical after the charge separation is suppressed by strong electrostatic repulsion induced by the adsorption of negatively charged $ZnTPPS_4$ on the CTAB cationic micellar surface or positively charged methyl viologen on the SDS anionic micellar surface. On the other hand, for Triton X-100, the charge recombination of the reduced methyl viologen and $ZnTPPS_4$ cation radical after the charge separation may be suppressed by complex formation of $ZnTPPS_4$ and Triton X-100 by hydrophobic effect.

3.5. Photoinduced hydrogen evolution with hydrogenase in various surfactant micelles

When the sample solution containing $ZnTPPS_4$, methyl viologen, triethanolamine, hydrogenase and surfactants was irradiated, hydrogen evolution was observed as shown in Fig. 7. The rate of hydrogen evolution in the presence of CTAB was about seven times as large as that in the absence of surfactants. For SDS, the effective photored-

Table 3
Lifetime of reduced methyl viologen and rate constant for back electron transfer, k_b in various surfactants

Surfactant	Lifetime of $MV^{+\cdot}$/ms	k_b/mol^{-1} dm^3 s^{-1}
None	0.020	8.9×10^9
CTAB (25 mmol dm^{-3})	2.3	5.7×10^7
Triton X-100 (40 mmol dm^{-3})	1.0	1.6×10^8
SDS [a] (25 mmol dm^{-3})	3.2	1.7×10^7

[a] Data from [8].

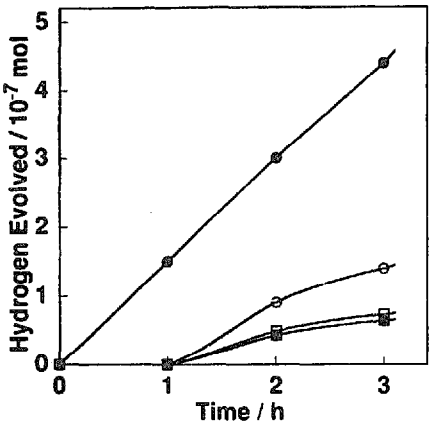

Fig. 7. Time dependence of hydrogen evolution. The sample solution containing triethanolamine (0.25 mol dm^{-3}), $ZnTPPS_4$ (0.13 μmol dm^{-3}), methyl viologen (0.22 mmol dm^{-3}) and hydrogenase (50 μl) in 25 mmol dm^{-3}) Tris–HCl buffer (pH = 7.4) was irradiated at 30°C. (■) without surfactant; (○) 40 mmol dm^{-3} Triton X-100; (●) 25 mmol dm^{-3} CTAB; (□) 25 mmol dm^{-3} SDS.

uction of methyl viologen was attained. However, little hydrogen evolution was observed by denaturation of hydrogenase by SDS [8,9]. On the other hand, in the presence of CTAB or Triton X-100, the increase of hydrogen evolution caused by effective electron transfer from reduced methyl viologen to hydrogenase was observed.

Acknowledgements

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