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Photoinduced hydrogen production with the system containing water-soluble viologen-linked porphyrins and hydrogenase

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Abstract

Water-soluble viologen-linked cationic porphyrins ($ZnP(C_nV)_4$, $n = 3$–6) and anionic porphyrins ($TPPSC_nV$, $n = 3$–6) were synthesized and their photochemical properties were characterized. The quenching processes of the photoexcited state of porphyrin moiety by the bonded viologen were measured by fluorescence lifetime and laser flash photolysis, and photoinduced hydrogen evolution with $ZnP(C_nV)_4$ or $TPPSC_nV$ and hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) was performed under steady state irradiation.

Keywords: Water-soluble viologen-linked porphyrins; Hydrogenase; Hydrogen production; Photoinduced electron transfer

1. Introduction

Photoinduced hydrogen evolution systems consisting of an electron donor (D), a photosensitizer (S), an electron carrier (C), and a catalyst have been used extensively for conversion of solar energy into chemical energy as shown in Scheme 1 [1–4].

In this reaction, charge separation between a photoexcited sensitizer and an electron carrier is one of the important steps. To improve this system, some viologen-linked zinc porphyrins (S–C) have been synthesized [5–8]. In the viologen-linked porphyrins, the photoexcited singlet state and the triplet state of porphyrin are easily quenched by the bonded viologen, compared with the viologen-free porphyrin. As viologen-linked porphyrins can act as both a photosensitizer and an electron carrier in the same molecule, these compounds were applied to photoinduced hydrogen evolution, as shown in Scheme 2.

Moreover, materials such as triethanolamine (TEOA) and ethylenediamine tetraacetic acid (EDTA) are sacrificial and are consumed when the photoreduction of water is carried out. Unlike these sacrificial reagents, NADPH can be a non-sacrificial electron donor. As shown in Scheme 3, oxidized NADPH (NADP, or D_{ox}) is easily photoreduced in the presence of grana, obtained from green plants. By combining reactions in Schemes 2 and 3, the splitting of water into hydrogen and oxygen is accomplished.

Thus, NADPH was useful as an electron-donating reagent in the photoinduced hydrogen evolution system.

In this work, a series of water-soluble viologen-linked cationic porphyrins ($ZnP(C_nV)_4$, $n = 3$–6)

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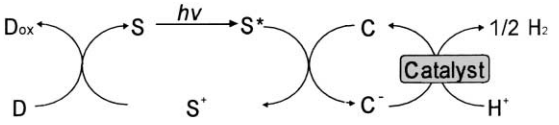
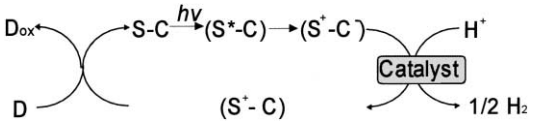
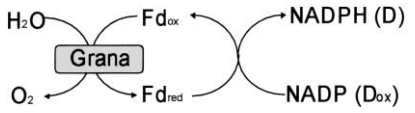


Scheme 1. Photoinduced hydrogen evolution system.



Scheme 2. Photoinduced hydrogen evolution using an electron carrier linked-photosensitizer (S–C).



Scheme 3. Photoinduced oxygen evolution system with grana-ferredoxin (Fd)-NADP.

and anionic porphyrins ($TPPSC_nV$, $n = 3$–6) with different methylene chain lengths ($n = 3$–6) between porphyrin and viologen, were synthesized and were characterized by using UV–VIS absorption spectra and fluorescence emission spectra. The quenching processes of the photoexcited singlet and triplet states of the porphyrin moiety of $ZnP(C_nV)_4$ and $TPPSC_nV$ by the bonded viologen were measured by using fluorescence lifetime and laser flash photolysis. These compounds were applied to photoinduced hydrogen evolution in a system containing nicotinamide–adenine dinucleotide phosphate (reduced form NADPH) as an electron donor, $ZnP(C_nV)_4$, or $TPPSC_nV$ and hydrogenase as a hydrogen evolution catalyst under steady state irradiation.

2. Experimental

2.1. Materials

5,10,15,20-Tetra(4-pyridyl)porphyrin (TPyP) was obtained from Aldrich Chemical Co., Inc. Tetraphenylporphyrin tetraslfonate (TPPS) was obtained from Dojin Laboratory. All the other materials were of the highest grade available.

2.2. Synthesis of water-soluble viologen-linked porphyrins

The structures of water-soluble viologen-linked cationic porphyrins ($ZnP(C_nV)_4$) and anionic porphyrins ($TPPSC_nV$) are shown in Fig. 1. $ZnP(C_nV)_4$ were prepared by the method reported previously [9]. The starting material, zinc-5,10,15,20-tetra(4-pyridyl)porphyrin (ZnTPyP), was synthesized according to the method described in the literature [10]. ZnTPyP was quaternized with an excess amount of 1-methyl-1-bromoalkyl-4,4′-bipyridinium (BrC_nCH_3) ($n = 3$–6) in dimethylformamide (DMF) at 90 °C for 48 h. The desired product was purified by gel column chromatography (Sephadex LH-20) without light.

$TPPSC_nV$ were prepared by the method reported previously [11]. The starting material, 5-(4-pyridyl)-10,15,20-triphenylporphyrin (PyTP), was synthesized according to the method described in the literature [12]. $TPPSC_nV$ was prepared as follows. PyTP was quaternized with an excess amount of α,ω-dibromoalkane (BrC_nBr) ($n = 3$–6) in toluene at 110 °C for 48 h to obtain 5-(4-bromoalkylpyridinium)-10,15,20-triphenylporphyrin ($TPPC_nBr$). $TPPC_nBr$ and an excess amount of 1-methyl-4,4′-bipyridinium were refluxed in DMF for 96 h to obtain viologen-linked porphyrin ($TPPC_nV$). The solvent was removed by vacuum pump. After washing with water and chloroform to remove unreacted 1-methyl-4,4′-bipyridinium and $TPPC_nBr$. A purple precipitate was collected by suction filtration. $TPPC_nV$ was refluxed with 20 ml of conc. H_2SO_4 for 4 h to obtain $TPPSC_nV$. After dilution with double-volume of water, the solution was added to acetone and then a green precipitate was collected by suction filtration and washed with acetone. The molecular structures of the synthesized compounds were characterized by ^{1}H-NMR (Varian GEMINI-200) [9,12].

2.3. Purification of hydrogenase

Hydrogenase from *Desulfovibrio vulgaris* (Miyazaki) was purified according to Yagi's method [13]. 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

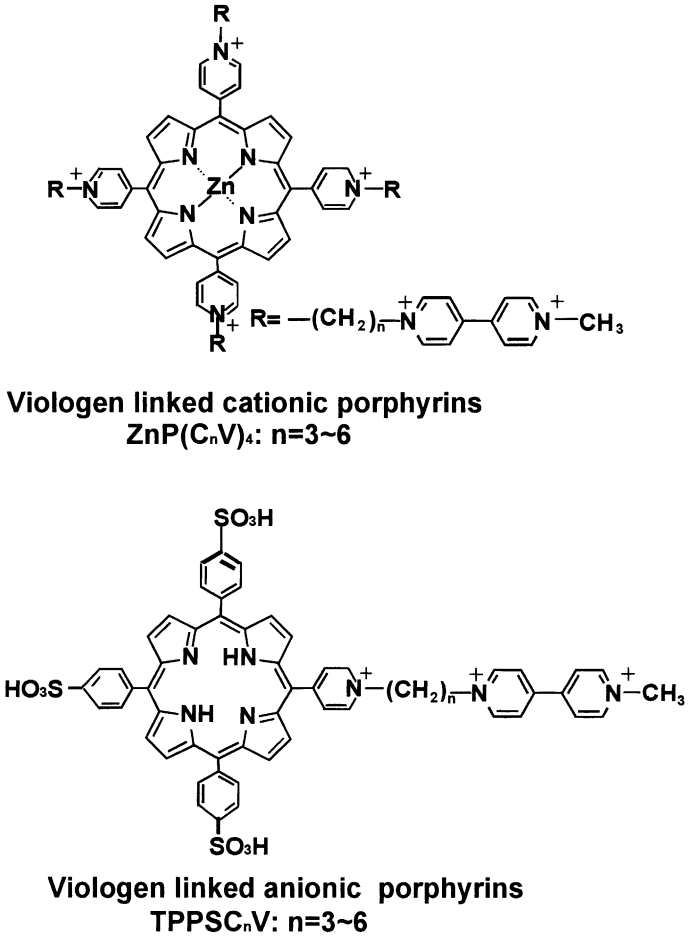


Fig. 1. Structures of water-soluble viologen-linked porphyrins.

(pH = 7.4) at 30 °C for 10 min. One unit of hydrogenase activity was defined as release 1.0 μmol hydrogen min^{-1}.

2.4. UV–VIS absorption spectra measurement

UV–VIS absorption spectra of $ZnP(C_nV)_4$ and $TPPSC_nV$ were recorded using Hitachi U-2000 spectrometer. The molar coefficients were determined by using the value of tetrakis-(4-methylpyridyl) zinc porphyrin (ZnTMPyP) for $ZnP(C_nV)_4$ and tetraphenylporphyrin tetraslfonate (TPPS) for $TPPSC_nV$.

2.5. Fluorescence emission spectra measurement

Fluorescence emission spectra of $ZnP(C_nV)_4$ and $TPPSC_nV$ were measured using Hitachi F-4010 spectrometer. The excitation wavelengths were 438 nm for $ZnP(C_nV)_4$ and 421 nm for $TPPSC_nV$. In these experiments, the absorbance at the excitation wavelength was kept constant to be 0.2 for all the sample solutions.

2.6. Fluorescence lifetime measurement

Fluorescence lifetime measurements were carried out by using time-correlated single-photon-counting

(Horiba NAES-500 spectrometer) at 25 °C. Fluorescence lifetimes were analyzed by deconvoluting the fluorescence decay curves by computer against the profile of excitation lamp decay at the excitation wavelength. After deconvolution of excitation lamp decay, fluorescence lifetimes were obtained by fitting with the following equation:

$$I(t) = \sum A_n \exp\left(-\frac{t}{\tau_n}\right), \quad (1)$$

where $I(t)$ is the time-resolved fluorescence, τ the fluorescence lifetime, and A_n is the fractional contributions to each lifetime component. The calculated values of $I_c(t)$ are then compared to the experimental $I(t)$ values; the goodness-of-fit is determined by minimizing the χ^2 function:

$$\chi^2 = \sum w_n[I(t) - I_c(t)]^2, \quad (2)$$

where w_n is a statistical weighting factor that accounts for the uncertainty in each $I(t)$ value. When w_n accurately describes the uncertainty in $I(t)$, χ^2 and the random distribution of residuals $[I_c(t) - I(t)]$ are equal to 1.0 and 0, respectively.

2.7. 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). The optical changes are given as $\Delta T/T_0$, where T_0 is the transmittance of the sample in the absence of the laser and T is the change produced by laser irradiation.

Decay curves were obtained by averaging 256 single shots, which were analyzed without deconvolution by fitting with the following equation:

$$T(t) = \sum C_n \exp\left(-\frac{t}{\tau_n}\right), \quad (3)$$

where $T(t)$ is the time-resolved transmittance, τ the lifetime of the photoexcited triplet state, and C_n is the fractional contributions to each lifetime component. The calculated values of $T_c(t)$ are then compared to the experimental $T(t)$ values; the goodness-of-fit is determined by minimizing the χ^2 function:

$$\chi^2 = \sum w_n[T(t) - T_c(t)]^2, \quad (4)$$

where w_n is a statistical weighting factor that accounts for the uncertainty in each $T(t)$ value. When w_n accurately describes the uncertainty in $T(t)$, χ^2 and the random distribution of residuals $[T_c(t) - T(t)]$ are equal to 1.0 and 0, respectively.

2.8. Photoreduction of the bonded viologen and photoinduced hydrogen production under 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. The sample solution containing NADPH (2.0 mmol dm^{-3}), $ZnP(C_nV)_4$ or $TPPSC_nV$ (2.5×10^{-6} mol dm^{-3}) and hydrogenase (0.35 unit) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4) was deaerated by repeated freeze–pump–thaw cycles and the evolved hydrogen was detected by gas chromatography (Shimadzu GC-14B; detector, TCD; column, active carbon).

2.9. Electrochemical measurements

Redox potentials were determined by cyclic voltammetry (Hokuto Denko Potentiostat/Galvanostat HA-301, Function Generation HB-111, Riken Densho X-Y recorder). All measurements were carried out under Ar in solutions 0.2 mol dm^{-3} of KCl and 25 mmol dm^{-3} Tris–HCl (pH = 7.4) at a carbon working electrode. A Pt was used as a counter electrode. All potentials are relative to Ag/AgCl electrode as the reference.

3. Results and discussion

3.1. UV–VIS absorption spectra of $ZnP(C_nV)_4$ and $TPPSC_nV$

Fig. 2(a) shows UV–VIS absorption spectra of $ZnP(C_3V)_4$ and $ZnP(C_3)_4$ as the example. The shape of absorption spectra of $ZnP(C_3V)_4$ is similar to

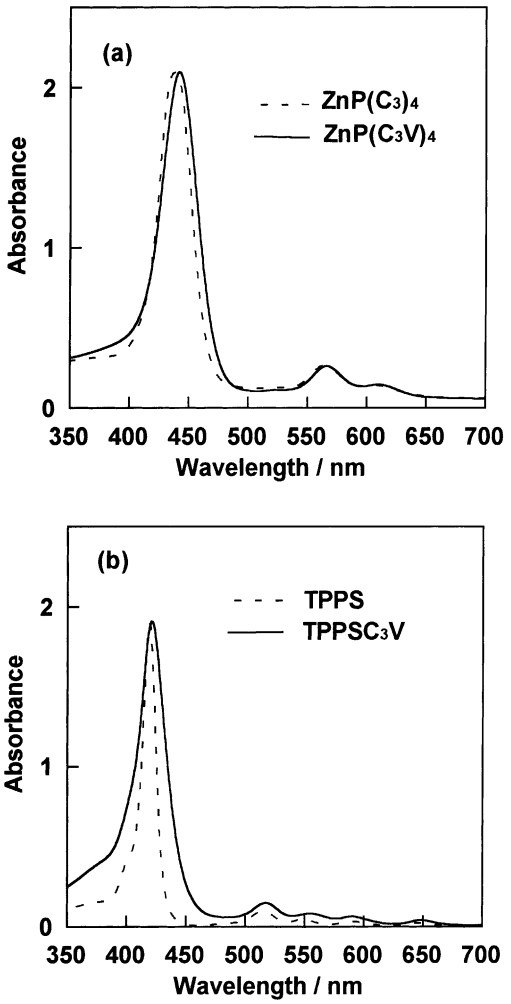


Fig. 2. UV–VIS absorption spectra of water-soluble viologen-linked porphyrins in water: (a) $ZnP(C_3V)_4$ (solid line) and $ZnP(C_3)_4$ (dash line); (b) $TPPSC_3V$ (solid line) and TPPS (dash line).

that of $ZnP(C_4)_4$. In the all cases of $ZnP(C_nV)_4$, the shapes of spectra are similar to that of viologen-free porphyrins. These results indicate no electronic interaction between the porphyrin site and the bonded viologen in $ZnP(C_nV)_4$ in the ground state. On the other hand, Fig. 2(b) shows UV–VIS absorption spectra of $TPPSC_3V$ and TPPS as the example. The peaks of absorption bands of $TPPSC_3V$ are almost the same values of viologen-free porphyrin TPPS. In the all cases of $TPPSC_nV$, the peaks of absorption bands are almost the same values of TPPS. However,

Table 1
The first excited singlet state energies of TPPS and redox potentials (versus Ag/AgCl) for TPPS and C_nVCH_3 and energies of the charge separated state

Compound	^{1}P (eV)[a]	E^1_o (V)[b]	E^1_r(V)[c]	$E(P^+V^-)$ (eV)[d]
TPPS	1.92	1.10		
$ZnP(C_3)_4$	1.99	0.958		
$ZnP(C_4)_4$	1.99	0.958		
$ZnP(C_5)_4$	1.99	0.958		
$ZnP(C_6)_4$	1.99	0.958		
C_3VCH_3			−0.662	
C_4VCH_3			−0.673	
C_5VCH_3			−0.682	
C_6VCH_3			−0.691	
$ZnP(C_3V)_4$	1.99			1.62
$ZnP(C_4V)_4$	1.99			1.63
$ZnP(C_5V)_4$	1.99			1.64
$ZnP(C_6V)_4$	1.99			1.65
$TPPSC_3V$	1.92			1.76
$TPPSC_4V$	1.92			1.77
$TPPSC_5V$	1.92			1.78
$TPPSC_6V$	1.92			1.79

[a] ^{1}P is the energy of the first excited singlet state taken as the average value of the frequencies of the longest wavelength of the absorption maxima and the shortest wavelength of the fluorescence emission maxima.
[b] E^1_o is the first oxidation potential.
[c] E^1_r is the first reduction potential.
[d] Calculated from the value of electrochemical measurement.

the Soret bands of $TPPSC_nV$ are broader than that of TPPS. This result shows electronic intermolecular or intramolecular interaction between the anionic porphyrin skeleton and the bonded viologen site in $TPPSC_nV$ in the ground state.

3.2. Electrochemical property of $ZnP(C_nV)_4$ and $TPPSC_nV$

The energy levels of $ZnP(C_nV)_4$ and $TPPSC_nV$ were studied by electrochemical measurements. The results are listed in Table 1. The energies of the first excited singlet states of $ZnP(C_nV)_4$ and $TPPSC_nV$ were calculated from the average value of the frequencies of the longest wavelength of absorption maxima and the shortest wavelength of the fluorescence emission maxima. The redox potentials were determined from cyclic voltammetric measurements. The energies of charge separated states of $ZnP^+(C_nV^-)_4$ and $TPPS^+C_nV^-$ were estimated from the first oxidation

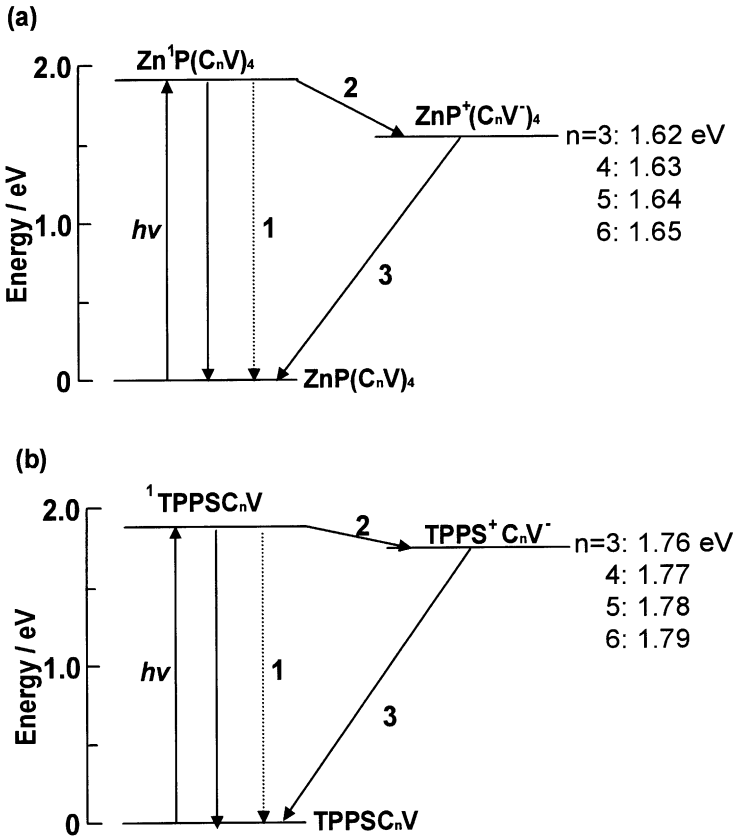


Fig. 3. (a) Energy levels of the first excited singlet state; and (b) charge separation state in $ZnP(C_nV)_4$ and $TPPSC_nV$.

potential of viologen-free porphyrin (ZnTMPyP for $ZnP(C_nV)_4$ and TPPS for $TPPSC_nV$) and the first reduction potential of C_nVCH_3. Each energy level is listed in Table 1. No correction for Coulomb effects was attempted because of any interaction between each chromphore at the ground state. For $ZnP(C_nV)_4$, the first excited singlet state of the porphyrin lies at 1.99 eV above the ground state and the $ZnP^+(C_nV^-)_4$ charge separated state lie at 1.62–1.65 eV, respectively. For $TPPSC_nV$, on the other hand, the first excited singlet state of the porphyrin lies at 1.92 eV above the ground state and the $TPPS^+C_nV^-$ charge separated state lie at 1.76–1.79 eV, respectively. Fig. 3(a) and (b) show the energy levels of transient states of $ZnP(C_nV)_4$ and $TPPSC_nV$ from Table 1, respectively. The electron transfer pathways in $ZnP(C_nV)_4$ and $TPPSC_nV$ are considered as shown in Fig. 3. Step 1 represents non-radiative and radiative processes. Step 2 represents electron transfer processes and step 3 represents charge recombination processes, respectively. The energy difference between the first excited singlet state of porphyrin site and the charge separated state is 0.34–0.37 eV for $ZnP(C_nV)_4$ and 0.13–0.16 eV for $TPPSC_nV$, respectively.

3.3. Fluorescence spectra of $ZnP(C_nV)_4$ and $TPPSC_nV$

The photoexcited singlet states of porphyrin moiety in $ZnP(C_nV)_4$ and $TPPSC_nV$ were investigated from emission spectra. Fig. 4(a) and (b) show typical fluorescence spectra of $ZnP(C_3V)_4$ and $TPPSC_3V$, respectively. Relative fluorescence intensities are summarized in Table 2. These values were obtained by integrating the emission spectra between 500 and 800 nm. The shape of fluorescence spectrum of

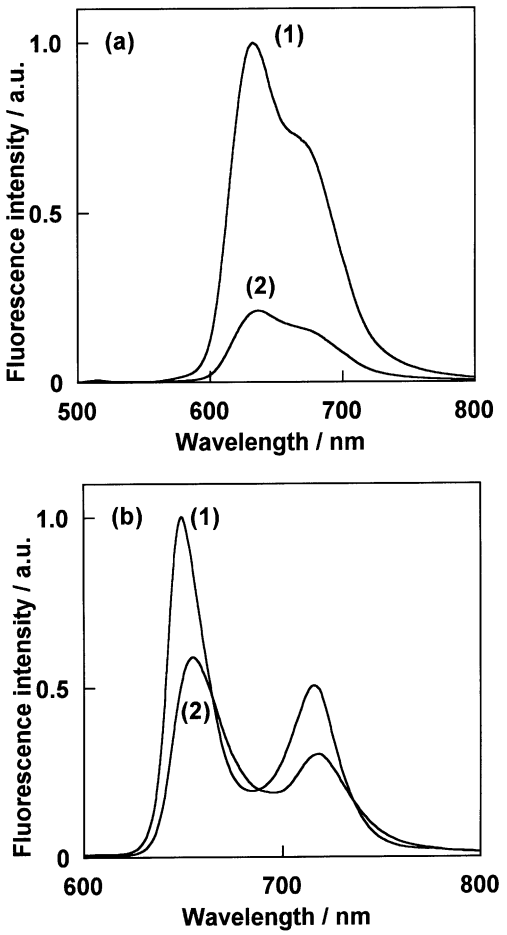


Fig. 4. Fluorescence spectra of water-soluble viologen-linked porphyrins in water: (a) $ZnP(C_3)_4$ (1) and $ZnP(C_3V)_4$ (2); (b) TPPS (1) and $TPPSC_3V$ (2). The excitation wavelengths for (a) and (b) was 438 and 421 nm, respectively.

$ZnP(C_3V)_4$ was the same as that of $ZnP(C_3)_4$, but the fluorescence intensity of $ZnP(C_3V)_4$ was lower than that of $ZnP(C_3)_4$. On the other hand, the shape of the spectrum of $TPPSC_3V$ also was the same as that of TPPS, but the fluorescence intensity of $TPPSC_3V$ was lower than that of TPPS. These results indicate that the photoexcited singlet state of the porphyrin site was quenched by the bonded viologen. In comparison of the fluorescence spectra of $TPPSC_nV$ and TPPS, the red-shift in the emission peak of $TPPSC_nV$ was observed. This result shows electronic intermolecular or intramolecular interaction between the photoexited singlet state of anionic porphyrin skeleton and the bonded viologen site in $TPPSC_nV$. The quenching of fluorescence of porphyrin moiety in $ZnP(C_nV)_4$ and $TPPSC_nV$ occurred by intramolecular electron transfer between the photoexcited singlet state of the porphyrin and the bonded viologen.

Table 2
Relative fluorescence intensities of $ZnP(C_nV)_4$ and $TPPSC_nV$[a]

Compound	I/I_0[b]
$ZnP(C_3V)_4$	0.20
$ZnP(C_4V)_4$	0.65
$ZnP(C_5V)_4$	0.73
$ZnP(C_6V)_4$	0.60
$TPPSC_3V$	0.58
$TPPSC_4V$	0.70
$TPPSC_5V$	0.56
$TPPSC_6V$	0.62

[a] Excitation wavelengths for $ZnP(C_nV)_4$ and $TPPSC_nV$ were 438 and 421 nm, respectively.

[b] I_0 is the fluorescence intensities of viologen-free porphyrin, $ZnP(C_n)_4$ for $ZnP(C_nV)_4$ and TPPS for $TPPSC_nV$.

3.4. Fluorescence lifetimes and electron transfer rate constants

Typical fluorescence decay profiles of $ZnP(C_3V)_4$ and $TPPSC_3V$ are shown in Fig. 4(a) and (b), respectively. The fluorescence decay of $ZnP(C_3)_4$ (1) and $ZnP(C_3V)_4$ (2) were multi-components. For $ZnP(C_3)_4$, the decay was not obtained the best-fit curves using Eqs. (1) and (2) in the time range of 0–50 ns. Thus, the decay was fitted at the initial stage of the decay (within 20 ns). The decay within 20 ns consisted of a single component and the fluorescence lifetime was 1.9 ns. For the other $ZnP(C_n)_4$, the decay was not obtained the best-fit curves using Eqs. (1) and (2) in the time range of 0–50 ns and the fluorescence decay within 20 ns also consisted of a single component and the fluorescence lifetime was 1.7–1.9 ns. For $ZnP(C_3V)_4$, the decay also was not obtained the best-fit curves using Eqs. (1) and (2) in the time range of 0–50 ns. Thus, the decay was fitted at the initial stage (within 20 ns). The decay within 20 ns consisted of the fluorescence decay of $ZnP(C_3V)_4$ consisted of two components and fluorescence lifetimes were 0.24 and 4.4 ns. For the other $ZnP(C_nV)_4$, the fluorescence decay and the fluorescence lifetimes of $ZnP(C_nV)_4$ also consisted of two components within 20 ns; a shorter lifetime (τ_s) and a longer lifetime (τ_l), as

Table 3
Fluorescence lifetime of $ZnP(C_nV)_4$ and $ZnP(C_n)_4$[a]

Compound	τ_s[b] (ns) (A_1 (%))	τ_l[b] (ns) (A_2 (%))	χ^{2c}
$ZnP(C_3V)_4$	0.24 (96.8)	4.4 (3.2)	1.13
$ZnP(C_3)_4$	1.9		1.15
$ZnP(C_4V)_4$	0.90 (91.5)	3.9 (8.5)	1.14
$ZnP(C_4)_4$	1.7		1.13
$ZnP(C_5V)_4$	0.90 (84.9)	3.0 (15.1)	1.13
$ZnP(C_5)_4$	1.8		1.13
$ZnP(C_6V)_4$	0.90 (84.5)	2.7 (15.5)	1.15
$ZnP(C_6)_4$	1.8		1.16

[a] Excitation wavelength was 350 nm.
[b] τ_s and τ_l were obtained by Eq. (1).
[c] χ^2 was obtained by Eq. (2).

shown in Table 3. The shorter lifetime of $ZnP(C_nV)_4$ was main component and its ratio was more than 80%. The longer lifetime component was large value compared with that of viologen-free zinc porphyrin by connection of viologen to zinc porphyrin. Connolly and coworkers [14] reported that two conformers exists in quinone-bonded porphyrins; complexed and extended conformers. Though the quinone comes close to the porphyrin moiety in a complexed conformer, the quinone is extended from the porphyrin moiety in an extended conformer. In the case of $ZnP(C_nV)_4$, there may be two conformers; a complexed conformer (the viologen is close to the porphyrin moiety) and extended conformers (the viologen is extended from the porphyrin moiety). The shorter lifetime and longer lifetime components in $ZnP(C_nV)_4$ are assigned to the complexed and extended conformer, respectively. On the other hand, the fluorescence decays of TPPS (1) and $TPPSC_3V$ (2) consisted of single components and fluorescence lifetimes were 12.7 and 9.1 ns, respectively. For the other $TPPSC_nV$, the fluorescence decay and the fluorescence lifetimes of $TPPSC_nV$ also consisted of a single component as shown in Table 4. The fluorescence lifetime of $TPPSC_nV$ was faster than that of TPPS, indicating that the photoexcited singlet state of the porphyrin was quenched by the bonded viologen. In the case of $TPPSC_nV$, there is not the complexed conformer and the extended conformers.

From the fluorescence lifetimes in Tables 3 and 4, intramolecular electron transfer rate constants (k_{et}) were estimated by the following equation:

$$k_{et} = \frac{1}{\tau_s} - \frac{1}{\tau_l}.$$

Table 4
Fluorescence lifetimes of TPPS and $TPPSC_nV$[a]

Compound	τ (ns)[b]	χ^{2c}
TPPS	12.7	1.09
$TPPSC_3V$	9.10	1.08
$TPPSC_4V$	10.4	1.10
$TPPSC_5V$	10.0	1.09
$TPPSC_6V$	10.1	1.08

[a] Excitation wavelength: 350 nm.
[b] τ_s and τ_l were obtained by Eq. (1). τ_l value was used the lifetime of TPPS.
[c] χ^2 was obtained by Eq. (2).

For $TPPSC_nV$, τ_s and τ_l is the fluorescence lifetimes of $TPPSC_nV$ and TPPS, respectively.

The results are shown in Table 5. For the $ZnP(C_nV)_4$, the intramolecular electron transfer rate in $ZnP(C_3V)_4$ is more rapid in comparison with the other $ZnP(C_nV)_4$. For the $TPPSC_nV$, on the other hand, the rate in $TPPSC_3V$ is more rapid compared with other $TPPSC_nV$. In $ZnP(C_3V)_4$ and $TPPSC_3V$, the distance between the porphyrin moiety and the bonded viologen is short and the electron transfer from the photoexcited singlet state of the porphyrin to the bonded viologen occurs easily.

3.5. Photoexcited triplet state of $ZnP(C_nV)_4$ and $TPPSC_nV$

The electron transfer from the photoexcited triplet state of porphyrin moiety to the bonded viologen was studied by using a laser flash photolysis. Fig. 5(a) shows a typical decay of photoexcited triplet state of porphyrin moiety of $ZnP(C_3)_4$ (1) and $ZnP(C_3V)_4$ (2) monitored at 490 nm of the maximum absorption of the photoexcited triplet state of the porphyrin moiety.

Table 5
Intramolecular electron transfer rate constant via the photoexcited singlet state, k_{et}, in $ZnP(C_nV)_4$ and $TPPSC_nV$

Compound	k_{et} (s^{-1})
$ZnP(C_3V)_4$	3.9×10^9
$ZnP(C_4V)_4$	8.5×10^8
$ZnP(C_5V)_4$	7.8×10^8
$ZnP(C_6V)_4$	8.8×10^8
$TPPSC_3V$	3.1×10^7
$TPPSC_4V$	1.7×10^7
$TPPSC_5V$	1.7×10^7
$TPPSC_6V$	1.7×10^7

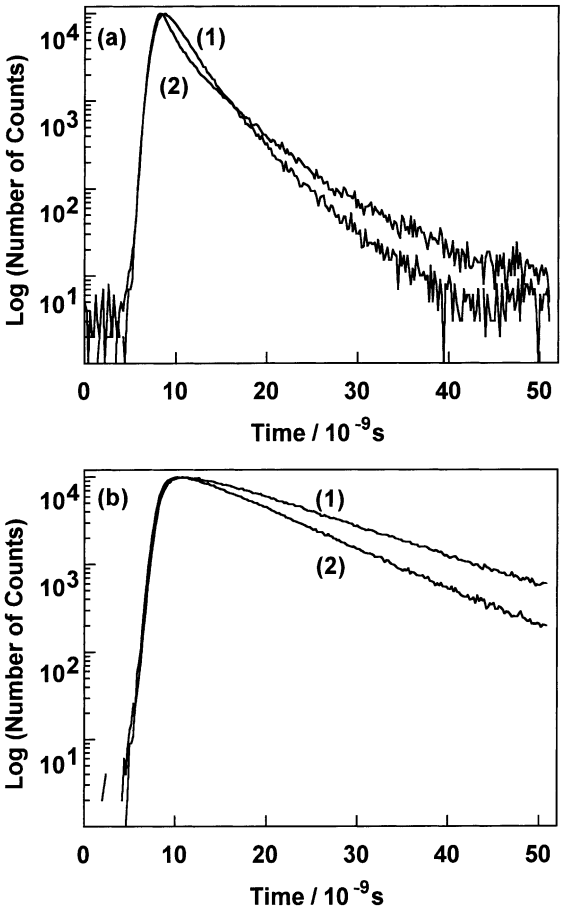


Fig. 5. Fluorescence decay curve of water-soluble viologen-linked porphyrins in water: (a) $ZnP(C_3)_4$ (1) and $ZnP(C_3V)_4$ (2); (b) TPPS (1) and $TPPSC_3V$ (2). The absorbance at the excitation wavelength (350 nm) was kept to be 0.4 for all the sample solutions.

The lifetime of photoexcited triplet state of porphyrin moiety of $ZnP(C_nV)_4$ and $ZnP(C_n)_4$ are summarized in Table 6. For all compounds, the best-fit curves and χ^2 value (nearly equal to 1.0) were obtained when n was equal to 1 in Eqs. (3) and (4). Thus, all the decays of $ZnP(C_nV)_4$ and $ZnP(C_n)_4$ obeyed first-order kinetics. From Fig. 5, the lifetimes of the photoexcited triplet state of $ZnP(C_3V)_4$ and $ZnP(C_3)_4$ were1.5 μs and 1.3 ms, respectively. In every case of $ZnP(C_nV)_4$, the lifetime of photoexcited triplet state of porphyrin moiety became shorter than that of $ZnP(C_n)_4$, indicating that the photoexcited triplet state of porphyrin moiety was quenched by the bonded viologen site. The initial amplitude of the transient absorption spectra of

Table 6
Lifetime of the photoexcited triplet state of $ZnP(C_nV)_4$, $ZnP(C_n)_4$, $TPPSC_nV$ and TPPS[a]

Compound	τ_{trip} (μs)[b]	χ^{2c}
$ZnP(C_3V)_4$	1.5	1.09
$ZnP(C_3)_4$	1300	1.10
$ZnP(C_4V)_4$	3.4	1.10
$ZnP(C_4)_4$	1300	1.11
$ZnP(C_5V)_4$	4.2	1.09
$ZnP(C_5)_4$	1400	1.08
$ZnP(C_6V)_4$	4.2	1.10
$ZnP(C_6)_4$	1300	1.11
TPPS	476	1.10
$TPPSC_3V$	403	1.09
$TPPSC_4V$	300	1.08
$TPPSC_5V$	300	1.10
$TPPSC_6V$	429	1.10

[a] Excitation wavelength was 532 nm.
[b] τ_{trip} was obtained by Eq. (3).
[c] χ^2 was obtained by Eq. (4).

the photoexcited triplet state of porphyrin moiety in $ZnP(C_nV)_4$ at 490 nm decreased compared with that of $ZnP(C_n)_4$. This result indicate that the intramolecular electron transfer rate via the photoexcited singlet state of porphyrin moiety from the bonded viologen site was faster than that of the rate of intersystem crossing from the photoexcited singlet state to the photoexcited triplet state of the porphyrin moiety. Thus, the yield of the photoexcited triplet state of the porphyrin moiety in $ZnP(C_nV)_4$ decreased compared with $ZnP(C_n)_4$. On the other hand, no increase of the absorbance at 605 nm, which is the characteristic absorption band of the reduced viologen, was observed. Thus, the intramolecular electron transfer via the photoexcited triplet state of porphyrin moiety did not occur in $ZnP(C_nV)_4$. Next, let us focus on the electron transfer reaction in the sample solution containing NADPH and $ZnP(C_nV)_4$ by using a laser flash photolysis. The increase of the absorbance at 605 nm was observed in sample solution containing NADPH and $ZnP(C_nV)_4$. The lifetime of the reduced viologen was estimated to be within 1.0 μs in all the $ZnP(C_nV)_4$. However, no increase of the absorbance at 605 nm was observed in sample solution containing triethanolamine (TEOA), instead of NADPH, and $ZnP(C_nV)_4$. As the bonded viologen was not reduced by NADPH, the photoexcited triplet state of porphyrin moiety was reduced by NADPH and then the bonded viologen was reduced by the electron transfer from reduced form of porphyrin

moiety. On the other hand, Fig. 5(b) shows a typical decay of photoexcited triplet state of porphyrin moiety of TPPS (1) and $TPPSC_3V$ (2) monitored at 470 nm of the maximum absorption of the photoexcited triplet state of the porphyrin moiety. The lifetime of photoexcited triplet state of porphyrin moiety of $TPPSC_nV$ and TPPS are summarized in Table 6. For all compounds, the best-fit curve and χ^2 value (nearly equal to 1.0) were obtained when n was equal to 1 in Eqs. (3) and (4). Thus, all the decays of $TPPSC_nV$ and TPPS obeyed first-order kinetics. From Fig. 5, the lifetimes of the photoexcited triplet state of $TPPSC_3V$ and TPPS were 403 and 476 μs, respectively. In every case of $TPPSC_nV$, the lifetimes of photoexcited triplet state of porphyrin moiety were almost the same compared with TPPS, indicating that the photoexcited triplet state of porphyrin moiety was not quenched by the bonded viologen. The initial amplitude of the transient absorption spectra of the photoexcited triplet state of porphyrin moiety in $TPPSC_nV$ at 470 nm decreased compared with that of TPPS. This result indicate that the intramolecular electron transfer rate via the photoexcited singlet state of porphyrin moiety from the bonded viologen site was faster than that of the rate of intersystem crossing from the photoexcited singlet state to the photoexcited triplet state of the porphyrin moiety. Thus, the yield of the photoexcited triplet state of the porphyrin moiety in $TPPSC_nV$ decreased compared with TPPS, and no increase of the absorbance at 605 nm, which is the characteristic absorption band of the reduced viologen, was observed. This result shows that the intramolecular electron transfer via the photoexcited triplet state of porphyrin moiety did not occur in $TPPSC_nV$. Next, let us focus on the electron transfer reaction in the sample solution containing NADPH or TEOA and $TPPSC_nV$ by using a laser flash photolysis. No increase of the absorbance at 605 nm was observed in sample solution containing NADPH or TEOA and $TPPSC_nV$. Thus, the photoexcited triplet state of porphyrin moiety and bonded viologen site were not quenched or reduced by these electron-donating reagents (Fig. 6).

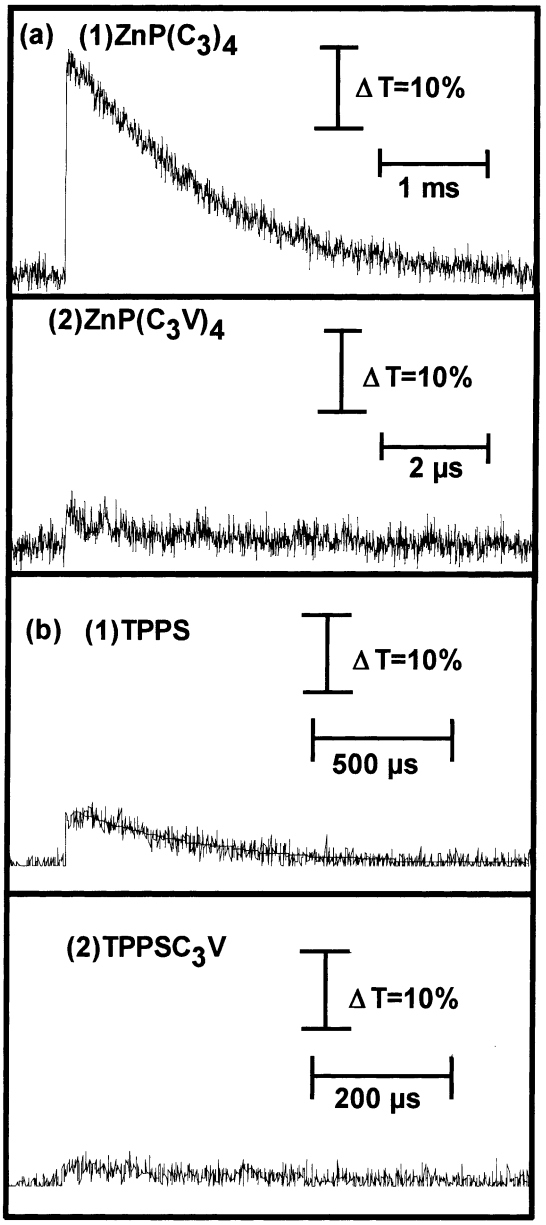


Fig. 6. Decay of the photoexcited triplet state of water-soluble viologen-linked porphyrins in water: (a) $ZnP(C_3)_4$ (1) and $ZnP(C_3V)_4$ (2) monitored at 490 nm; (b) TPPS (1) and $TPPSC_3V$ (2) monitored at 470 nm. The absorbance at the excitation wavelength (532 nm) was kept to be 0.2 for all the sample solutions. ΔT is transmittance of the photoexcited triplet state of porphyrin moiety.

3.6. Photoreduction of viologen site of $ZnP(C_nV)_4$ and $TPPSC_nV$

When the sample solution containing NADPH and $ZnP(C_nV)_4$ or $TPPSC_nV$ was irradiated, the spectrum

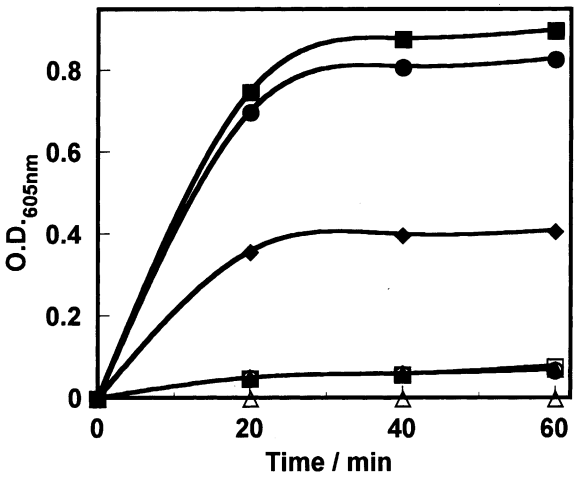


Fig. 7. The time dependence of the change of absorbance at 605 nm attributed to the reduced viologen moiety under steady state irradiation. The sample solution consisting of NADPH (2.0 mmol dm^{-3}) and $ZnP(C_nV)_4$ or $TPPSC_nV$ (2.5×10^{-6} mol dm^{-3}) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4): NADPH (2.0 mmol dm^{-3}), ZnTMPyP (2.5×10^{-6} mol dm^{-3}), and methylviologen (1.0×10^{-5} mol dm^{-3}) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4) (◆); NADPH (2.0 mmol dm^{-3}), TPPS (2.5×10^{-6} mol dm^{-3}), and methylviologen (2.5×10^{-6} mol dm^{-3}) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4) (△); $ZnP(C_3V)_4$ (○); $ZnP(C_4V)_4$ (●); $ZnP(C_5V)_4$ (■); $ZnP(C_6V)_4$ (□); $TPPSC_6V$ (▲).

of $ZnP(C_nV)_4$ or $TPPSC_nV$ changed with time. The time dependence of the change of absorbance at 605 nm attributed to the reduced viologen moiety is shown in Fig. 7. For $ZnP(C_nV)_4$, the change of absorption at 605 nm is due to the reduced viologens increased and the absorption spectrum intensity with 438 nm is due to the Soret band of the porphyrin decreased by the irradiation. This result indicates that the reduced form of the porphyrin may be formed by the reductive quenching of the photoexcited triplet state of the porphyrin by NADPH and then the bonded viologens are reduced. When TEOA was used as an electron donor instead of NADPH, the change of absorption at 605 nm is due to the reduced viologen and the absorption intensity with 438 nm is due to the Soret band of the porphyrin were not observed by the irradiation. Thus, photoreduction rate of the bonded viologen site of $ZnP(C_nV)_4$ depend on the nature of the electron-donating reagents. The photoexcited triplet state of $ZnP(C_nV)_4$ was quenched or reduced by NADPH. On the other hand, the triplet state was not quenched or reduced by TEOA. These results indicate that the reduced form of the porphyrin may be formed by the reductive quenching of the photoexcited triplet state of the porphyrin by NADPH and then the bonded viologens are reduced. By using $TPPSC_nV$ except $TPPSC_6V$, on the other hand, the change of absorption at 605 nm attributed to the reduced viologen was not observed. For $TPPSC_6V$, on the other hand, the change of absorption at 605 nm increased and the absorption spectrum intensity with 421 nm is due to the Soret band of the porphyrin did not decrease by the irradiation. When TEOA was used as an electron donor instead of NADPH, the change of absorption at 605 nm increased and the absorption intensity with 421 nm did not decrease by the irradiation. Thus, photoreduction rate of the bonded viologen site of $TPPSC_6V$ was independent of the nature of the electron-donating reagents. The photoexcited triplet state of $TPPSC_6V$ was not quenched or reduced by NADPH or TEOA. From the result of laser flash photolysis, the photoexcited triplet state of porphyrin was not quenched by the bonded viologen site. These results indicate that the bonded viologens are reduced by the electron transfer via the photoexcited singlet state of porphyrin.

3.7. Photoinduced hydrogen evolution with hydrogenase

When the sample solution containing NADPH, $ZnP(C_nV)_4$ or $TPPSC_nV$, and hydrogenase was irradiated, the time dependence of hydrogen evolution was observed as shown in Fig. 8. $ZnP(C_nV)_4$ can be substrates of the hydrogenase. In the case of $ZnP(C_4V)_4$ (■) or $ZnP(C_5V)_4$ (●), the higher hydrogen evolution rate was observed than that of an individual component system (◆) consisting of ZnTMPyP, NADPH, methylviologen and hydrogenase. When methylene chain length (*n*) is 4 or 5, the porphyrin moiety come to close enough to viologen by a conformational change of the molecule, so that the electron may transfer directly from the porphyrin to viologen. By using $TPPSC_nV$ except $TPPSC_6V$, on the other hand, no hydrogen evolution was observed. In the case of $TPPSC_6V$ (▲), the higher hydrogen evolution rate was observed than that of an individual component system (△) consisting of TPPS, NADPH, methylviologen and hydrogenase. When TEOA was used as an electron donor instead of NADPH, little hydrogen

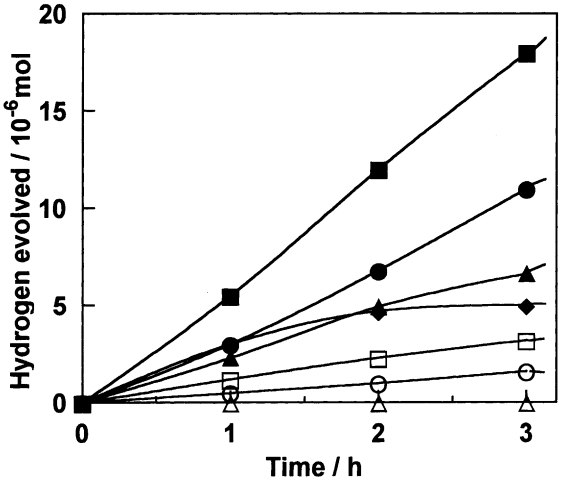


Fig. 8. Time dependence of hydrogen evolution under steady state irradiation. The sample solution consisting of NADPH (2.0 mmol dm^{-3}), $ZnP(C_nV)_4$ or $TPPSC_nV$ (2.5×10^{-6} mol dm^{-3}), and hydrogenase (0.35 unit) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4): NADPH (2.0 mmol dm^{-3}), ZnTMPyP (2.5×10^{-6} mol dm^{-3}), methylviologen (1.0×10^{-5} mol dm^{-3}), and hydrogenase (0.35 unit) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4) (◆); NADPH (2.0 mmol dm^{-3}), TPPS (2.5×10^{-6} mol dm^{-3}), methylviologen (2.5×10^{-6} mol dm^{-3}), and hydrogenase (0.35 unit) in 4.0 ml of 25 mmol dm^{-3} Tris–HCl (pH = 7.4) (△); $ZnP(C_3V)_4$ (○); $ZnP(C_4V)_4$ (●); $ZnP(C_5V)_4$ (■); $ZnP(C_6V)_4$ (□); $TPPSC_6V$ (▲).

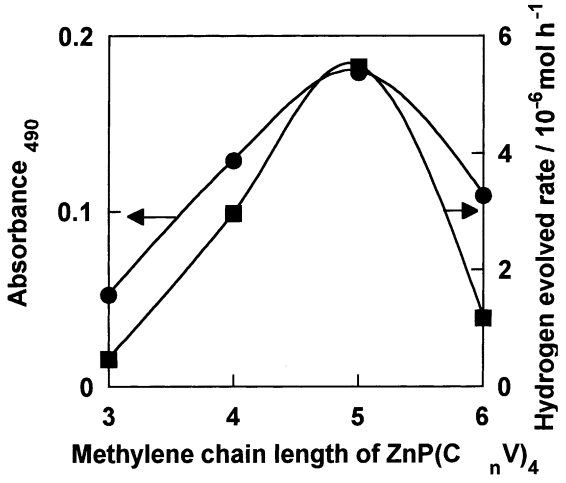


Fig. 9. The relationship of absorption of the photoexcited triplet state of $ZnP(C_nV)_4$ and the initial rate of hydrogen evolution. The absorption of the photoexcited triplet state of $ZnP(C_nV)_4$ was measured at 490 nm: Absorbance of the photoexcited triplet state of $ZnP(C_nV)_4$ (●); initial rate of hydrogen evolution with hydrogenase and $ZnP(C_nV)_4$ (■).

evolution was observed in $ZnP(C_nV)_4$. In the case of $TPPSC_6V$, on the other hand, hydrogen evolution was observed using TEOA instead of NADPH. These results supported the data of the photoreduction of viologen site of $ZnP(C_nV)_4$ and $TPPSC_nV$.

3.8. Mechanism of photoinduced hydrogen evolution using $ZnP(C_nV)_4$ and $TPPSC_nV$

The mechanisms of photoinduced hydrogen evolution using $ZnP(C_nV)_4$ and $TPPSC_nV$ were proposed by the results of kinetic parameters obtained from fluoresce lifetime measurement, laser flash photolysis, photoreduction of the bonded viologen and photoinduced hydrogen evolved rate by steady state irradiation. For the $ZnP(C_nV)_4$, at the first stage of the photoreduction, the photoexcited triplet state of the porphyrin is reductively quenched by the electron donor, as the porphyrin used in this experiment is cationic. When $ZnP(C_nV)_4$ was irradiated in the presence of NADPH, the absorption spectrum intensity with 438 nm due to the Soret band of the porphyrin decreased, indicating the reduced form of the porphyrin may be formed by the reductive quenching of the photoexcited triplet state of the porphyrin by NADPH. On the other hand, the bonded viologen was not reduced by NADPH. As shown in Fig. 9, the absorbance of the photoexcited triplet state of $ZnP(C_nV)_4$ strongly depends on the methylene chain length and the absorbance has the correlation of the photoinduced hydrogen evolution activity. The photoexcited triplet state of $ZnP(C_nV)_4$ corresponded to the initial hydrogen evolution rate. This result indicates that hydrogen evolution proceeded via the photoexcited triplet state of $ZnP(C_nV)_4$. The proposed mechanism is as shown in Fig. 10(a). In the first step, the photoexcited singlet state of $ZnP(C_nV)_4$ is formed by the irradiation. Then the photoexcited triplet state of $ZnP(C_nV)_4$ is formed by intercrossing reaction in the second step (step 4). In the third, the porphyrin moiety is reduced by quenching of photoexcited triplet state with NADPH (step 6), and finally the electron transfer from the reduced porphyrin moiety to the viologen is occurred (step 7) and hydrogen evolved by electron transfer from reduced viologen to hydrogenase (step 8). From the fluorescence lifetime decay

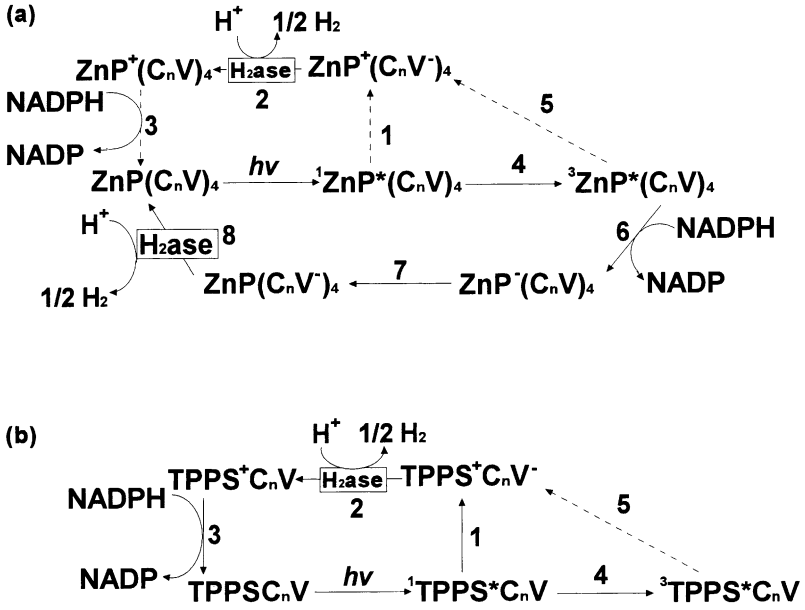


Fig. 10. Possible mechanism of photoinduced hydrogen evolution with hydrogenase and $ZnP(C_nV)_4$ (a) and $TPPSC_nV$ (b).

measurements, the electron transfer from the photoexcited singlet state of porphyrin to the bonded viologen was very rapid (step 1). This result indicates the no photoinduced hydrogen evolution occurs via the photoexcited singlet state of porphyrin (steps 1–3). When $TPPSC_6V$ was irradiated in the presence of NADPH, on the other hand, the absorption spectrum intensity with 421 nm due to the Soret band of the porphyrin did not change, indicating the oxidized form of the porphyrin may be formed by the oxidative quenching of the photoexcited singlet state of the porphyrin by bonded viologen. The proposed mechanism is as shown in Fig. 10(b). In the first step, the photoexcited singlet state of $TPPSC_nV$ is formed by the irradiation. The electron transfer from the photoexcited singlet state of porphyrin moiety to the viologen is occurred (step 1) in the second step, and finally hydrogen evolved by electron transfer from reduced viologen to hydrogenase (step 2) and the oxidized porphyrin site is reduced by NADPH (step 3). The photoexcited triplet state of $TPPSC_nV$ is formed by intercrossing reaction in the second step (step 4). From the result of laser flash photolysis, the photoexcited triplet state of porphyrin was not quenched by the bonded viologen site. Thus, the electron transfer from the photoexcited triplet state of porphyrin moiety to the viologen does not occur (step 5).

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