BCSJ Award Article

Photoinduced Hydrogen-Evolution System with an Antibody–Porphyrin Complex as a Photosensitizer

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A complex between a monoclonal antibody for porphyrin and zinc–porphyrin was utilized to construct an energy conversion system. Monoclonal antibody 2B6 bound *meso*-tetrakis(4-carboxyphenyl)porphyrin zinc complex (ZnTCPP) with a dissociation constant of 2.1×10^{-8} M. Upon binding the antibody, the lifetime of the excited triplet state of ZnTCPP increased from 0.5 to 1.2 ms. A stable cationic radical of viologen was obtained by irradiating the solution containing the complex of 2B6 with ZnTCPP, methyl viologen (MV^{2+}), and ethylenediaminetetraacetic acid tetrasodium salt (EDTA-4Na) with light. When colloidal platinum was added as a catalyst, photoinduced hydrogen production was observed upon continuous irradiation of visible light. The estimated turnover number of photoinduced hydrogen evolution was 5.0×10^{-3} s^{-1}. The catalytic activity of the 2B6–ZnTCPP complex on the hydrogen evolution was compared with that of ZnTCPP alone and the complexes of ZnTCPP with fragments of antibody 2B6 (2B6-H and 2B6-L). The heavy chain of antibody 2B6 mainly contributed to the complex formation with ZnTCPP and the resultant hydrogen production, and the whole antibody–ZnTCPP complex led to the efficient hydrogen production.

Recently, the design of new energy sources has received much attention because the exhaustion of fossil fuels has become a pressing issue. Furthermore, combustion of fossil fuels causes emission of CO_2, which has resulted in global warming. Hence, solar energy has become popular due to its limitlessness. Moreover energy derived from hydrogen has received a lot of attention as a clean energy source. Currently, there are many attempts to create energy conversion systems, which convert solar energy into chemical energy.[1] On the other hand plants and light harvesting bacteria convert solar energy into chemical energy with high efficiency.[2]

In the field of artificial energy conversion, hydrogen production systems by photoelectrolysis of water constructed using TiO_2 electrodes have been developed.[3] However, TiO_2 absorbs only UV light, and it cannot use the major part of sunlight. To overcome this problem, dye-sensitized hydrogen-evolution systems have been developed.[4] It is advisable to mimic the energy conversion systems in nature, and the chromophores of in vivo photosynthetic reaction centers are fixed by the protein environment.[5] The distances between electron donors and electron acceptors are noncovalently held under optimum conditions. Recently, non-covalently assembled donor–acceptor arrays have been constructed utilizing hydrogen bonding,[6] metal coordination,[7] apoproteins,[8] electrostatic interactions,[9] and supramolecular formations.[10]

To construct a photoinduced hydrogen production system, a suitable catalyst is necessary to reduce a proton by an electron, which can be obtained by charge separation. In many cases, colloidal platinum has been used as a catalyst for proton reduction.[11] To construct an artificial charge-separation system with non-covalently linked electron donors and acceptors, it is favorable to use proteins as the matrices for electron donors and acceptors as in vivo photosynthetic reaction centers. Monoclonal antibodies are thought to be suitable as artificial protein matrices due to their high selectivity and affinity.[12] Incorporation of electron donors into the binding site of monoclonal antibodies can be expected to give a specific environment for electron donors to lengthen their excited state lifetimes and to regulate interactions between donors and acceptors. We have been preparing monoclonal antibodies for porphyrins, and using these complexes of antibodies with synthetic porphyrins to construct artificial electron-transfer systems.[13]

In this study, monoclonal antibodies for 5,10,15-tris(4-carboxyphenyl)-20-[4-(2,5-dihydroxybenzylcarbamoyl)phenyl]-porphyrin (TCPP-HQ, Figure 1)[14] were prepared. One of the monoclonal antibodies, 2B6, enhanced the photoinduced electron transfer from *meso*-tetrakis(4-carboxyphenyl)porphyrin zinc complex (ZnTCPP) to methyl viologen (MV^{2+}) by complex formation with ZnTCPP. A stable cationic radical of viologen was obtained by photoirradiating the solution containing the complex of 2B6 with ZnTCPP, MV^{2+}, and

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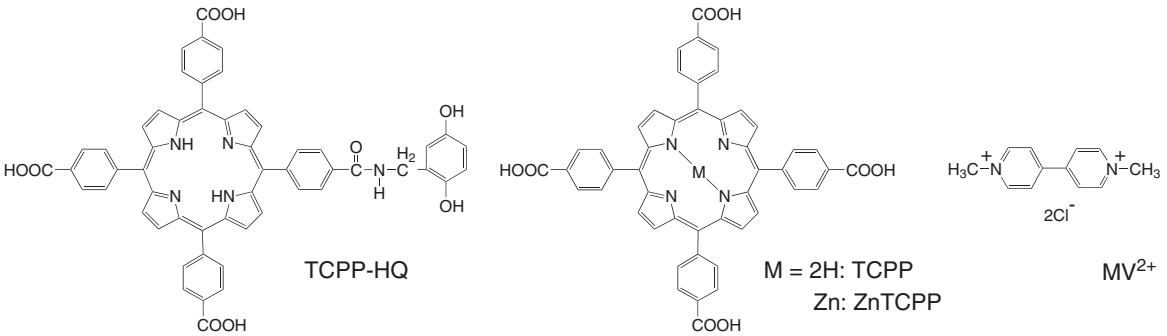
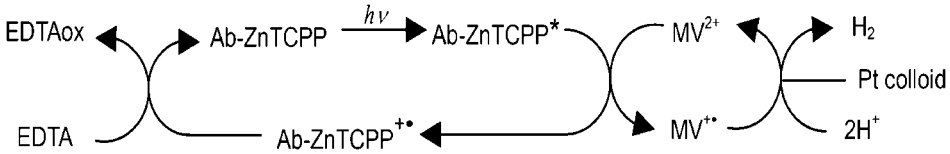


Figure 1. Structures of TCPP-HQ, TCPP, ZnTCPP, and MV^{2+}.



Scheme 1. Hydrogen-evolution system utilizing a monoclonal antibody–porphyrin complex as a photosensitizer.

ethylenediaminetetraacetic acid tetrasodium salt (EDTA-4Na). Hence, we constructed a hydrogen-evolution system using monoclonal antibodies for porphyrins where the complex of antibody 2B6 with ZnTCPP was used as a photosensitizer and colloidal platinum as a catalyst (Scheme 1). The amount of hydrogen gas production was monitored in the presence of ZnTCPP alone, 2B6–ZnTCPP complex, and the mixture of ZnTCPP with fragmented antibody 2B6 (2B6-H or 2B6-L).

Results and Discussion

Complex Formation of Monoclonal Antibody 2B6 with ZnTCPP. Monoclonal antibody 2B6 (IgG_1) was prepared using TCPP-HQ as a hapten. Antibody 2B6 bound not only TCPP, but also ZnTCPP with dissociation constants of 2.0×10^{-8} and 2.1×10^{-8} M, respectively. The ground state interaction of the antibody with ZnTCPP caused a peak shift in the region of the Soret band of ZnTCPP toward a longer wavelength. The fluorescence intensity of ZnTCPP increased upon complexation with antibody 2B6. The addition of the antibody increased the lifetime of excited triplet state (^{3}ZnTCPP*) from 0.5 to 1.2 ms, indicating that a bimolecular triplet–triplet annihilation process was inhibited by the binding of the antibody to ZnTCPP.

Electron Transfer from ZnTCPP to MV^{2+}. MV^{2+} was used as an electron acceptor for ZnTCPP. A transient absorption spectroscopic study was performed to investigate electron transfer from the excited state of ZnTCPP to MV^{2+} in the presence of the antibody as shown in Figure 2a. The lifetime of ^{3}ZnTCPP* in the 2B6–ZnTCPP complex decreased from 1.2 ms to 0.4 μs upon the addition of MV^{2+}, while the absorption of the cationic radical of methyl viologen ($MV^{+\bullet}$) appeared around 610 nm. Furthermore, the absorption of ^{3}ZnTCPP* decreased (Figures 2b and 2c), which corresponded with the increase in $MV^{+\bullet}$. These results show that MV^{2+} was reduced to $MV^{+\bullet}$ by electron transfer from ^{3}ZnTCPP* to MV^{2+}. The lifetime of ^{3}ZnTCPP* without 2B6 was 0.2 μs in the presence of MV^{2+}. Hence, the electron transfer from

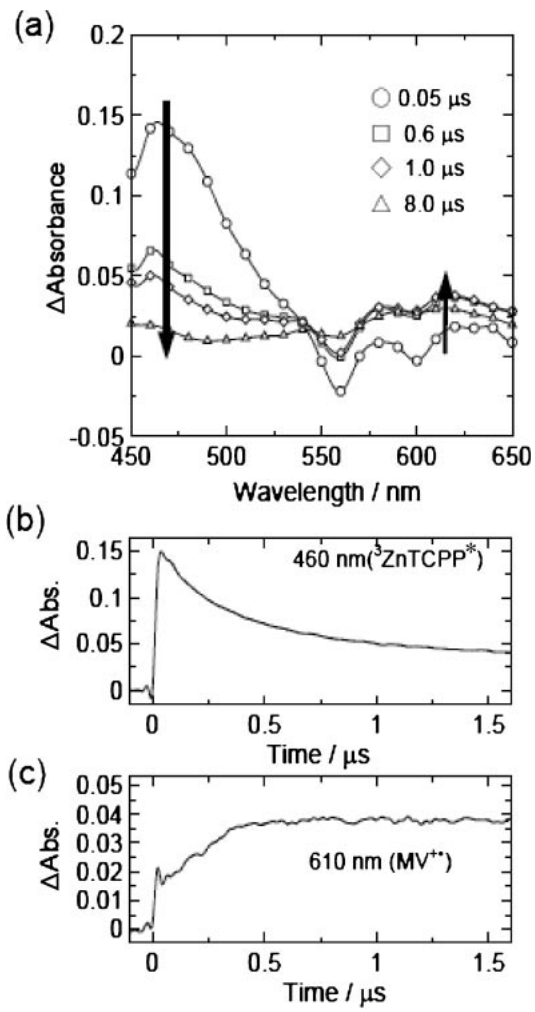


Figure 2. Transient absorption spectra (a) and time profiles of ^{3}ZnTCPP* (b) and $MV^{+\bullet}$ (c) in the presence of antibody 2B6. $\lambda_{ex} = 532$ nm, $[ZnTCPP] = 1.0 \times 10^{-5}$ M, [antibody 2B6] $= 1.0 \times 10^{-5}$ M, and $[MV^{2+}] = 2.0 \times 10^{-3}$ M in 0.1 M phosphate borate buffer (PBB, pH 9.0).

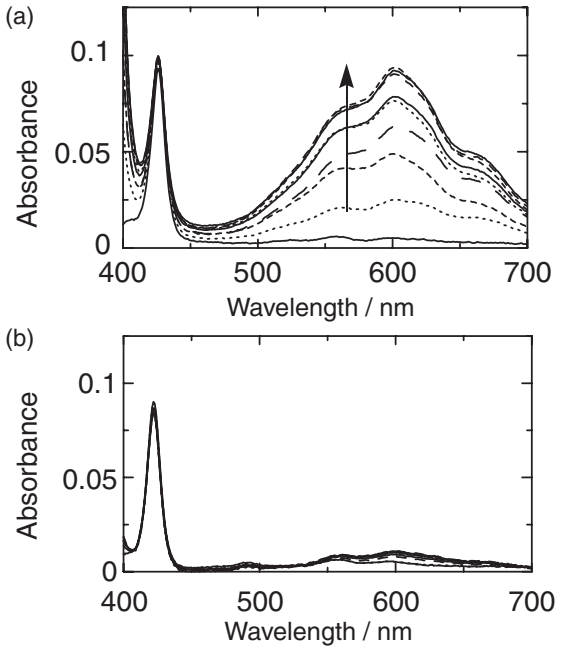


Figure 3. UV–vis spectral changes of the solutions of the mixture of ZnTCPP and MV^{2+} recorded at 5 min intervals upon irradiating with light in the presence (a) and absence (b) of antibody 2B6. [ZnTCPP] = 2.5×10^{-7} M, [antibody 2B6] = 2.5×10^{-7} M (a) and 0 M (b), [MV^{2+}] = 1.0×10^{-4} M, and [EDTA-4Na] = 1.0×10^{-2} M in 0.1 M PBB (pH 9.0).

^{3}ZnTCPP* in the binding site of 2B6 to MV^{2+} was slower than that without antibodies.

Photoreduction of MV^{2+}. Figure 3a shows the production of $MV^{+\bullet}$ by continuously irradiating the solution containing antibody 2B6, ZnTCPP, MV^{2+}, and ethylenediaminetetraacetic acid tetrasodium salt (EDTA-4Na) as a sacrifice electron donor with visible light. The absorption around 602 nm increased with irradiation time. Based on the molar extinction coefficient of MV^+, the estimated amount of $MV^{+\bullet}$ produced was 7.5 μM after 45 min of light irradiation,[15] whereas the amount of $MV^{+\bullet}$ produced in the absence of 2B6 was 0.4 μM (Figure 3b). The quantity of $MV^{+\bullet}$ generated in the 2B6–ZnTCPP system was 19 times larger than that with ZnTCPP alone. The binding of 2B6 to ZnTCPP likely suppressed the back electron transfer in the charge-separated state, leading to the efficient production of $MV^{+\bullet}$. $MV^{+\bullet}$ was catalytically generated by a photoinduced electron transfer from ZnTCPP in the complex with 2B6 to MV^{2+}. Figure 4 shows the amounts of $MV^{+\bullet}$ produced upon 45 min of light irradiation at various concentrations of the ZnTCPP–antibody 2B6 complex. The total concentration of $MV^{+\bullet}$ increased in proportion to that of the complex. The turnover number of the 2B6–ZnTCPP complex on the catalytic $MV^{+\bullet}$ production was 1.3×10^{-2} s^{-1}.

Binding Properties of the Whole Antibody 2B6 or Fragmented 2B6 to ZnTCPP, and the Photoinduced Electron-Transfer Behavior. The photoinduced $MV^{+\bullet}$ production in the presence of fragmented antibodies was also examined to further consider the effect of protein binding to ZnTCPP on the production of $MV^{+\bullet}$. The antigen binding site of antibodies consists of two peptide chains: heavy chain (H-chain) and light chain (L-chain). Antibody 2B6 was divided to its H- and L-chain fragments by reduction of the disulfide bonds of the antibody with dithiothreitol (=1,4-dimercapto-2,3-butanediol). The dissociation constants of the complexes between antibody fragments (2B6-H and 2B6-L) and ZnTCPP were determined by the fluorescence changes derived from the tryptophan residue of the antibody fragments by the addition of ZnTCPP. The estimated K_d value of the complex between 2B6-H and ZnTCPP was 5.0×10^{-8} M. On the other hand, affinity of 2B6-L to ZnTCPP was not observed. Hence, the H-chain of antibody 2B6 mainly contributed to complexation with ZnTCPP. To investigate the environmental difference around ZnTCPP in the complexes with whole antibody 2B6 or 2B6-H, spectral changes of the Soret band of ZnTCPP upon the addition of antibody 2B6 and 2B6-H were measured. The peak shift of the Soret band of ZnTCPP upon complexation of ZnTCPP with 2B6 was 4 nm (423 to 427 nm), which is attributed to the incorporation of a ZnTCPP molecule into the hydrophobic environment of the binding site of 2B6. Upon complexation between ZnTCPP and 2B6-H, the Soret band of ZnTCPP shifted 2 nm (423 to 425 nm). This small shift demonstrated fragmentation of antibody 2B6 into its H- and L-chains weakened the interaction between ZnTCPP and the antibody. Figure 5 shows the UV–vis spectra of ZnTCPP at various concentrations of MV^{2+} in the presence of whole antibody 2B6 (a), 2B6-H (b), and absence of antibodies (c). The degree of spectral changes of ZnTCPP by the addition of MV^{2+} was in the order of without antibodies > with 2B6-H > with whole antibody 2B6, suggesting that the binding site of 2B6-H has rather an open structure relative to that of whole antibody 2B6. MV^{2+} molecules easily approach the ZnTCPP molecule in the binding site of antibody 2B6-H. Figure 6 shows the amount of $MV^{+\bullet}$ produced by irradiating the solution of ZnTCPP, MV^{2+}, EDTA-4Na, with and without antibodies with visible light. The amount of $MV^{+\bullet}$ produced in the absence of antibody 2B6 was only 0.4 μM after 45 min of light irradiation. On the other hand, 7.5 μM of $MV^{+\bullet}$ was produced in the presence of whole antibody 2B6. This amount was larger than that in the 2B6-H–ZnTCPP system (5.7 μM). Therefore, binding of whole antibody 2B6 to ZnTCPP

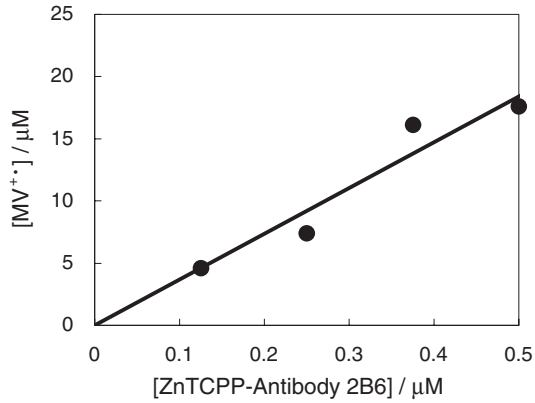


Figure 4. Amount of $MV^{+\bullet}$ produced by 45 min light irradiation at various concentrations of ZnTCPP–2B6 complex. [MV^{2+}] = 1.0×10^{-4} M and [EDTA-4Na] = 1.0×10^{-2} M in PBB.

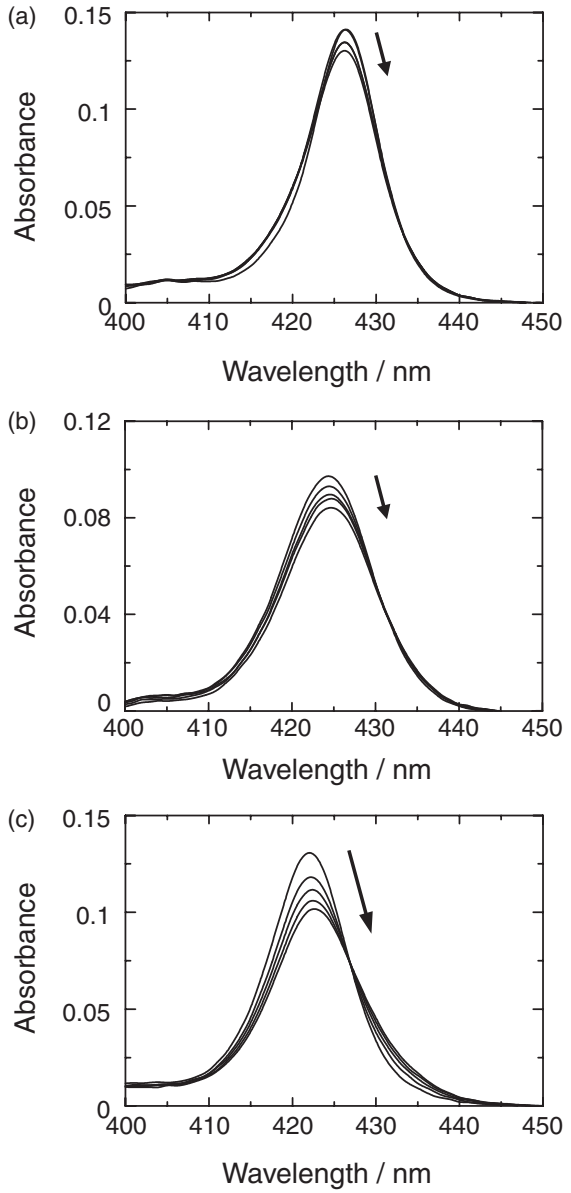


Figure 5. UV–vis spectral changes of ZnTCPP by adding MV^{2+} in the presence of whole antibody 2B6 (a), 2B6-H (b), and absence of antibodies (c). [ZnTCPP] = 2.5×10^{-7} M, [2B6 (binding site) or 2B6-H] = 5.0×10^{-7} M (a or b), [MV^{2+}] = 0, 25, 50, 75, and 100 μM, and [EDTA-4Na] = 1.25×10^{-3} M.

provided a preferable environment around ZnTCPP to efficiently produce $MV^{+\bullet}$.

Hydrogen Production by Light Irradiation. We constructed a hydrogen-evolution system using the 2B6–ZnTCPP complex, MV^{2+}, and colloidal Pt as a catalyst. An increase in the concentration of hydrogen was observed upon irradiating a solution with the above components with light (Figure 7). The reduction of water catalytically proceeded to give 18 μM of hydrogen gas after 60 min of light irradiation in the presence of antibody 2B6. The 2B6–ZnTCPP complex had a turnover number of $5.0 \times 10^{-3}\,s^{-1}$ on the hydrogen evolution, and it was ca. eight times higher than that of ZnTCPP without antibodies ($6.3 \times 10^{-4}\,s^{-1}$).

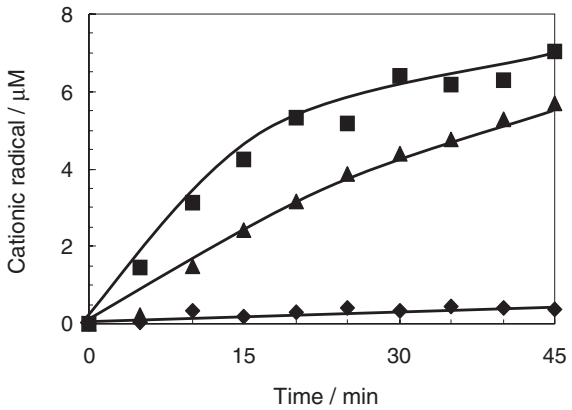


Figure 6. Generation of $MV^{+\bullet}$ by continuously irradiating solutions of ZnTCPP and MV^{2+} with 2B6 (■), 2B6-H (▲), and without antibodies (◆) with visible light. [ZnTCPP] = 2.5×10^{-7} M, [2B6 (binding site) or 2B6-H] = 5.0×10^{-7} M (■ or ▲) and 0 M (◆), [MV^{2+}] = 1.0×10^{-4} M, and [EDTA-4Na] = 1.0×10^{-2} M.

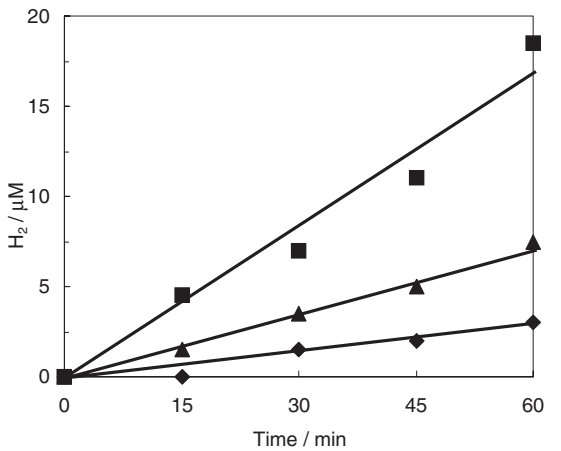


Figure 7. Concentrations of hydrogen gas generated under steady-state irradiation of visible light (wavelength >400 nm) in the presence of 2B6 (■), 2B6-H (▲), and without antibodies (◆). [ZnTCPP] = 1.0×10^{-6} M, [the binding site of 2B6 or 2B6-H] = 2.0×10^{-6} M (■ or ▲) and 0 M (◆), [MV^{2+}] = 5.0×10^{-4} M, [colloidal Pt] = 1.2×10^{-6} M, and [EDTA-4Na] = 1.0×10^{-2} M.

The photoinduced hydrogen production in the presence of antibody 2B6-H was also monitored under the same conditions. The amount of hydrogen produced after irradiating with light for 60 min was 7.0 μM in the 2B6-H–ZnTCPP system. Additionally, the complex between 2B6-H and ZnTCPP was found to have catalytic activity for the photosensitized reduction of water to hydrogen, but the efficiency of hydrogen evolution in the complex of whole antibody 2B6 with ZnTCPP was the highest among the systems examined in this study. The concentration of hydrogen produced from water was proportional to the catalytic activity of these complexes to generate $MV^{+\bullet}$ as well as the binding affinity of the protein (2B6 or 2B6-H) to ZnTCPP.

Other papers have reported attempts to construct photoinduced hydrogen-evolution systems using porphyrin–protein

complexes as a photosensitizer, for example, a complex of recombinant human serum albumin (HSA) with Zn protoporphyrin IX,[11f] reconstituted myoglobin (Mb) with a synthetic Zn porphyrin,[8g] and light-harvesting chlorophyll–protein complex of photosystem II (LHCII).[11j] The turnover numbers of these systems, HSA–Zn porphyrin, Mb–Zn porphyrin, and LHCII, were estimated to be 2.6×10^{-3}, 1.0×10^{-3}, and $2.8 \times 10^{-3}\,s^{-1}$, respectively. On the other hand, our system using the whole antibody–Zn porphyrin complex had a higher turnover, $5.0 \times 10^{-3}\,s^{-1}$. All systems had a catalytic activity on the same order of magnitude, but the 2B6–ZnTCPP complex prepared in this study had the highest activity in the photoproduction of hydrogen among artificial photosensitizing protein–porphyrin complexes.

Conclusion

We prepared monoclonal antibody 2B6, which binds ZnTCPP with a dissociation constant of 2.1×10^{-8} M. The lifetime of the excited triplet state of the ZnTCPP increased from 0.5 to 1.2 ms upon complexation with the antibody. Photoinduced electron transfer from ^{3}ZnTCPP* to MV^{2+} was observed by measuring the transient absorption spectra of ZnTCPP and $MV^{+\bullet}$. In the presence of the 2B6–ZnTCPP complex, a stable electron-transfer product was obtained, and a hydrogen-evolution system was constructed by utilizing the complex of 2B6 with ZnTCPP, MV^{2+}, and colloidal Pt. The amount of hydrogen produced in the presence of antibody 2B6 was eight times larger than that without antibodies. It is remarkable that the efficiency of hydrogen production in the system utilizing the complex of whole antibody with ZnTCPP is the highest among artificial photosensitizing protein–porphyrin complexes.

Experimental

Materials and Apparatus. ZnTCPP was synthesized according to the literature.[13a] MV^{2+} was purchased from Nacalai Tesque Inc., and used as received in the measurements. Additional chemicals were purchased from Nacalai Tesque Inc., Tokyo Chemical Industry Co., Ltd., and Wako Pure Chemical Industries, Ltd. Colloidal platinum was prepared according to the literature.[16] The preparation procedure of monoclonal antibody 2B6 was described in our previous report.[14] UV–vis spectra were recorded on a Shimadzu UV-2100 UV–visible spectrophotometer, while fluorescence spectra were recorded on a Hitachi F-2500 fluorescence spectrophotometer.

Separation of Antibody 2B6 into Heavy and Light Chains. The heavy chains and light chains of antibody 2B6 were separated by reduction of the disulfide bonds of the antibody with dithiothreitol.[17] To an aqueous solution of antibody 2B6 (6.6×10^{-6} M), ethylenediaminetetraacetic acid tetrasodium salt (EDTA-4Na, 1.0 mM) was dissolved. Dithiothreitol (0.1 M) in 0.5 mL of tris buffer (pH 7.0) was added to the solution. Then the solution was deaerated by bubbling Ar gas, and the solution was allowed to react for 1 h. Iodoacetic acid (0.2 M) in 0.5 mL of tris buffer was added and stirred overnight. The heavy chains and light chains of antibody 2B6 were purified using a protein A column (Ampure PA kit, Amersham Pharmacia Biotech). The purity of these chains was confirmed by SDS-PAGE.

Determination of the Dissociation Constants of the Complexes between Antibody 2B6 or Fragmented Antibody 2B6 and ZnTCPP. Dissociation constants (K_d) of the complexes between antibodies and porphyrins were determined by the Scatchard plot as follows:[18]

$$i/[\text{porphyrin}] = 1/K_d - i/K_d \quad (1)$$

where [porphyrin] shows the concentration of free porphyrin, i is the fraction of the antibody–porphyrin complex determined by the fluorescence quenching of antibodies upon addition of porphyrins.

Measurement of Transient Absorption Spectra. Transient absorption spectra of ZnTCPP were monitored by a Nd^{3+}/YAG laser spectroscopy system; the second harmonic at 532 nm was used for excitation. A digitizing oscilloscope (Hewlett Packard HP 54522, 500 MHz) was used to digitize the photocurrent of the photomultiplier. The concentrations of ZnTCPP, the binding sites of 2B6, and MV^{2+} were set at 1.0×10^{-5}, 1.0×10^{-5}, and 1.0×10^{-3} M, respectively. The solvent was a phosphate borate buffer (PBB, 0.1 M, pH 9.0). All samples were deaerated by Ar bubbling for 15 min prior to the measurements.

Hydrogen Evolution. ZnTCPP (1.0×10^{-6} M), 2B6 or 2B6-H (binding site, 2.0×10^{-6} M), MV^{2+} (5.0×10^{-4} M), colloidal Pt (1.2×10^{-6} M), and EDTA-4Na (1.0×10^{-2} M) were mixed in 2 mL of 0.1 M buffer. The sample solution was deaerated by Ar bubbling for 20 min. The solution was irradiated with a 500 W Xe lamp (Ushio UI-501C) where wavelengths shorter than 400 nm were cut off using a glass filter (GG-400; Schott Glass Technologies, Inc.). The amount of the generated hydrogen was monitored by a hydrogen sensor (DM-10B2; ABLE) attached to the cuvette. Control experiments were performed for the solution containing ZnTCPP, MV^{2+}, colloidal Pt, and EDTA-4Na without antibodies.

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