J|A|C|S
ARTICLES
Published on Web 11/30/2006

Photosensitized Reduction of Water to Hydrogen Using Human Serum Albumin Complexed with Zinc−Protoporphyrin IX

Teruyuki Komatsu,*[,†,‡] Rong-Min Wang,[†,§] Patricia A. Zunszain,[¶] Stephen Curry,[¶] and Eishun Tsuchida*[,†]

Contribution from the Advanced Research Institute for Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan, PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi-shi, Saitama 332-0012, Japan, Key Laboratory of Polymer Materials of Gansu Province, Northwest Normal University, Lanzhou 730070, China, and Biophysics Section, Division of Cell and Molecular Biology, Faculty of Natural Sciences, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom

Received August 5, 2006; E-mail: teruyuki@waseda.jp; eishun@waseda.jp

Abstract: We present the photophysical properties of complexes of recombinant human serum albumin (rHSA) with Zn(II)−protoporphyrin IX (ZnPP) and their activities in the photosensitized reduction of water to hydrogen (H_2) using methyl viologen (MV^{2+}) as an electron relay. The ZnPP is bound in subdomain IB of wild-type rHSA [rHSA(wt)] by an axial coordination of Tyr-161 and, in the rHSA(I142H/Y161L) mutant [rHSA(His)], by a His-142 coordination. Both the rHSA(wt)−ZnPP and rHSA(His)−ZnPP complexes showed a long-lived photoexcited triplet state with lifetimes (τ_T) of 11 and 2.5 ms, respectively. The accommodation of ZnPP into the protein matrix efficiently eliminated the collisional triplet self-quenching process. The addition of a water-soluble electron acceptor, MV^{2+}, resulted in a significant decrease in the triplet lifetime. The transition absorption spectrum revealed the oxidative quenching of rHSA−^{3}ZnPP* by MV^{2+}. The quenching rate constant (k_q) and backward electron transfer rate constant (k_b) were determined to be 1.4×10^7 and 4.7×10^8 M^{-1} s^{-1} for rHSA(wt)−ZnPP. In the presence of the colloidal PVA−Pt as a catalyst and triethanolamine (TEOA) as a sacrificial electron donor, the photosensitized reduction of water to H_2 takes place. The efficiency of the photoproduction of H_2 was greater than that of the system using the well-known organic chromophore, tetrakis(1-methylpyridinium-4-yl)porphinatozinc(II) ($ZnTMPyP^{4+}$), under the same conditions.

Introduction

The photosensitized reduction of water to molecular H_2, which is a clean-burning fuel free of CO_2 emission, has attracted considerable attention during the past decade. In order to trigger this reaction by visible light, organic chromophores are extensively used as photosensitizers, such as ruthenium tris(bipyridyl) complexes and zinc−porphyrins.[1,2] The classical, but effective system, appears to consist of water-soluble, positively charged tetrakis(1-methylpyridinium-4-yl)porphinatozinc(II) ($ZnTMPyP^{4+}$), methyl viologen (MV^{2+}), a colloidal Pt catalyst, and sacrificial electron donor.[1a,2] Instead of the synthetic $ZnTMPyP^{4+}$, if one can use the most prominent porphyrin in nature, namely, protoporphyrin IX, it would have a significant impact not only on pure chemistry but also on solar energy conversion. However, the Zn(II) complex of protoporphyrin IX (ZnPP) is relatively insoluble in water (<pH 9), and it is therefore difficult to employ ZnPP in order to construct a practical catalyst system in aqueous media.

Human serum albumin (HSA), the most abundant plasma protein in our bloodstream, acts as a transporter for a range of insoluble endogenous and exogenous compounds, such as fatty acids, bilirubin, thyroxine, hemin [Fe(III)−protoporphyrin IX], and a variety of drugs.[3,4] This heart-shaped monomer protein contains three homologous domains (I−III), each of which is composed of A and B subdomains.[4,5] Recent X-ray crystallographic studies have revealed that hemin is bound within a narrow hydrophobic D-shaped cavity in subdomain IB of HSA with an axial coordination of Tyr-161 to the central ferric

[†] Waseda University.
[‡] JST.
[§] Northwest Normal University.
[¶] Imperial College London.

(1) (a) Kalyanasundaram, K.; Grätzel, M. *Helv. Chim. Acta* **1980**, *63*, 478−485. (b) Kalyanasundaram, K. *Coord. Chem. Rev.* **1982**, *46*, 159−244.
(2) (a) Harriman, A.; Porter, G.; Richoux, M.-C. *J. Chem. Soc., Faraday Trans. 2* **1981**, *77*, 833−844. (b) Richoux, M.-C.; Harriman, A. *J. Chem. Soc., Faraday Trans. 1* **1982**, *78*, 1873−1885. (c) Harriman, A.; Porter, G.; Richoux, M.-C. *Coord. Chem. Rev.* **1982**, *44*, 83−126.
(3) Peters, T. *All about Albumin: Biochemistry, Genetics and Medical Applications*; Academic Press: San Diego, CA, 1996.
(4) (a) Curry, S.; Madelkow, H.; Brick, P.; Franks, N. *Nat. Struct. Biol.* **1998**, *5*, 827−835. (b) Petitpas, I.; Petersen, C. E.; Ha, C.-E.; Bhattacharya, A. A.; Zunszain, P. A.; Ghuman, J.; Bhagavan, N. V.; Curry, S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6440−6445. (c) Ghuman, J.; Zunszain, P. A.; Petitpas, I.; Bhattacharya, A. A.; Otagiri, M.; Curry, S. *J. Mol. Biol.* **2005**, *353*, 38−52.
(5) (a) He, X. M.; Carter, D. C. *Nature* **1992**, *358*, 209−215. (b) Carter, D. C.; Ho, J. X. *Adv. Protein Chem.* **1994**, *45*, 153−203.

ion.[6,7] Furthermore, we have demonstrated that site-directed mutagenesis to introduce a proximal histidine into position Ile-142 and to replace Tyr-161 by Leu at the heme-binding site [rHSA(I142H/Y161L); rHSA(His)] confers a reversible dioxygen binding capability to the prosthetic heme group in a fashion similar to hemoglobin.[8]

In this paper, we report for the first time the photophysical properties of rHSA complexes with a ZnPP [rHSA(wt)−ZnPP, rHSA(His)−ZnPP] and their photoinduced electron transfer to MV^{2+} and highlight their activities for the photosensitized reduction of water to H_2 in the presence of colloidal PVA−Pt as a catalyst and triethanolamine (TEOA) as a sacrificial regent.

Experimental Section

Materials and Apparatus. All reagents were purchased from commercial sources as special grades and used without further purification. The rHSA(wt) and rHSA(I142H/Y161L) mutant [rHSA-(His)] were prepared according to our previously reported procedures.[7,8] Zinc(II)−protoporphyrin IX (ZnPP) was purchased from Sigma-Aldrich. 5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)porphinatozinc(II) tetrachloride ($ZnTMPyP^{4+}$) was synthesized by insertion of the central zinc(II) into 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphin tetra-*p*-toluenesulfonate (Sigma-Aldrich) using $Zn(AcO)_2$ followed by exchanging the counteranions with chlorides using a Bio-Rad AG 1-X8 resin (100−200 mesh) chloride form with CH_3CN/H_2O (1/1). The UV−vis absorption spectra were recorded using an Agilent 8453 UV−visible spectrophotometer with an Agilent 89090A temperature control unit. The fluorescence spectra were obtained from a HITACHI F-4500 spectrofluorometer. Water was deionized using a Millipore Elix and Simpli Lab-UV.

Preparation of rHSA(wt)−ZnPP and rHSA(His)−ZnPP. Typically, 5 mL of a potassium phosphate buffered solution (pH 7.0, 50 mM) of rHSA(wt) (0.1 mM) was mixed with 0.8 mL of 0.625 mM ZnPP in DMSO (ZnPP:rHSA molar ratio of 1:1) and incubated for 12 h with rotation in the dark at room temperature. The complex was then diluted with 50 mM potassium phosphate and concentrated to the initial volume using a Vivaspin 20 centrifuge filter (10 kDa MW cutoff) at 4000*g* using a Beckman Coulter Allegra X-15R centrifuge. These dilution/concentration cycles were repeated to reduce the DMSO concentration to <0.1 vol %. The phosphate buffered solution (pH 7.0, 10 mM) of rHSA(wt)−ZnPP ([ZnPP] = 10 μM) in a 10 mm path length optical quartz cuvette sealed with a rubber septum was degassed and purged with Ar prior to use. The rHSA(His)−ZnPP solution was prepared by the same procedure.

Excited State Lifetimes. The singlet lifetimes of rHSA−ZnPP were measured using a HORIBA NAES-500 nanosecond fluorometer with a N_2 lamp (excitation side: an Asahi spectra MZ0560 multicavity filter ($\lambda = 560 \pm 2$ nm), emission side: a HOYA R-620 sharp cut filter). The samples (5 μM) were held in a quartz cuvette (optical path length = 10 mm), and the experiments were carried out at 25 °C.

The transient absorption spectra and triplet lifetime measurements were carried out using a Unisoku TSP-1000WK time-resolved spectrophotometer with a Spectron Laser Systems SL803G-10 Q-switched Nd:YAG laser, which generated a second-harmonic (532 nm) pulse of 6 ns duration (10 Hz).[8b]

Photoreduction of MV^{2+}. Steady-state irradiations were carried out using an Oriel 450 W xenon arc lamp model 66021 in conjunction with an Asahi Spectra MZ0550 multicavity filter (550 ± 2 nm). The filtered light (1.61 $mWcm^{-2}$) was used to irradiate the potassium phosphate buffered solution (pH 5.4, 50 mM) of rHSA−ZnPP (10 μM, 3.5 mL) including MV^{2+} (2 mM) and TEOA (0.19 M) as it was gently stirred in a 10 mm path length quartz cuvette under an Ar atmosphere at 25 °C. The distance between the cuvette and the light source was 12 cm.

The overall quantum efficiency (Φ) of the photoreduction of MV^{2+} was calculated as the ratio (numbers of $MV^{+\bullet}$ molecules produced)/(numbers of photons absorbed); the denominator was determined by the change in the power of the transmitted light, measured by an Advantest Q8230 photo power meter with a Q82313 silicon photodiode sensor, which was placed after the quartz cuvette.

Hydrogen Evolution from Water. Colloidal PVA−Pt was prepared as follows. An aqueous H_2PtCl_6 solution (66 mM, 1 mL) was added dropwise to an $EtOH/H_2O$ (1/1) solution (100 mL) of poly(vinyl alcohol) (PVA, 271 mg, average degree of polymerization = 500, Wako Pure Chemical Industries) with vigorous stirring, and the mixture was refluxed for 1.5 h. After being cooled to room temperature, a dark brown PVP−Pt colloid solution was obtained. To remove the EtOH and produce the aqueous colloidal PVP−Pt, the solution was evaporated using a rotary evaporator at 50 °C. The concentration was assayed based on the Pt ion by inductively coupled plasma (ICP) spectrometry using a Seiko Instruments SPS 7000A spectrometer; [Pt−PVA] = 3.55 mM. The diameter of the colloidal Pt was determined to be approximately 3.0 ± 0.7 nm using transmission electron microscopy observations on a JEOL JEM 1011 transmission electron microscope.

The phosphate buffered solution (pH 5.4, 50 mM, 3.5 mL) of the mixture of rHSA−ZnPP (10 μM), MV^{2+} (2 mM), TEOA (0.19 M), and a colloidal PVA−Pt (20 μM) in a quartz cuvette (optical path length = 10 mm) connected to a 40 mL glass chamber was degassed by Ar. Steady-state irradiations were carried out at 25 °C using a 450 W xenon arc lamp in conjunction with a HOYA HA30 heat absorption filter (330−700 nm) to eliminate the UV light and excess heating. The distance between the cuvette and light source was 12 cm. After a designated period, 100 μL of the gas above the solution was removed by a gastight syringe and applied to a Shimadzu GC-8APT gas chromatograph with a TCD detector to measure the amount of H_2. The column used was a Shinwa Chemical Industries Shincarbon ST.

Results and Discussion

Photophysical Properties of rHSA−ZnPP Complexes. The rHSA(wt)−ZnPP and rHSA(His)−ZnPP complexes were prepared essentially as described previously for HSA−hemin complexes.[8] The gel permeation chromatogram (Superdex 75g) of the pink-colored proteins exhibited only a single elution peak, indicating that ZnPP is perfectly incorporated into rHSA. No precipitation was observable for over a year at 4 °C. Our crystal structure analysis and spectroscopy experiments revealed that hemin is bound within a D-shaped hydrophobic cavity in subdomain IB of rHSA(wt) with a relatively weak axial coordination by Ty-161.[7,8] In the genetically engineered rHSA-(His) mutant, hemin is in the ferric high-spin complex with an axial His-142 coordination and a water molecule as the sixth ligand.[8b]

The UV−vis absorption spectra of the phosphate buffered solution (pH 7.0, 10 mM) of rHSA(wt)−ZnPP showed a Soret band at 420 nm and the Q-bands at 547 and 585 nm (Figure 1A), which are very similar to those for the toluene solution of Zn(II)−protoporphyrin IX dimethylester (ZnPPDME) with 5 vol % ethanol (Figure 1B). The aqueous rHSA(His)−ZnPP solution exhibited the same spectral features of ZnPPDME-coordinated 1-methylimidazole (1-MIm) in toluene. The bandwidth at half-height of the Soret band [$\Delta\lambda_{1/2}$ = 21 nm for rHSA(wt)−ZnPP or 19 nm for rHSA(His)−ZnPP] was identical to that observed

(6) Wardell, M.; Wang, Z.; Ho, J. X.; Robert, J.; Ruker, F.; Rubel, J.; Carter, D. C. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 813−819.
(7) Zunszain, P. A.; Ghuman, J.; Komatsu, T.; Tsuchida, E.; Curry, S. *BMC Struct. Biol.* **2003**, *3*, 6.
(8) (a) Komatsu, T.; Ohmichi, N.; Zunszain, P. A.; Curry, S.; Tsuchida, E. *J. Am. Chem. Soc.* **2004**, *126*, 14304−14305. (b) Komatsu, T.; Ohmichi, N.; Nakagawa, A.; Zunszain, P. A.; Curry, S.; Tsuchida, E. *J. Am. Chem. Soc.* **2005**, *127*, 15933−15942.

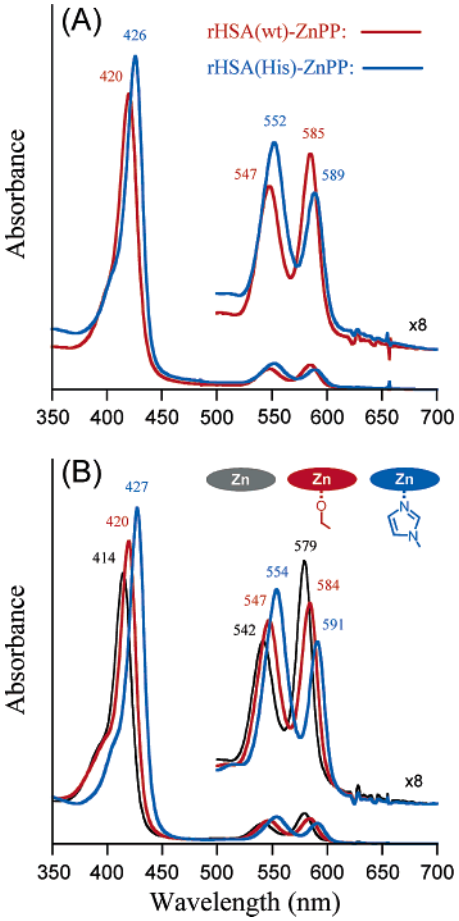


Figure 1. (A) UV−vis absorption spectra of rHSA(wt)−ZnPP and rHSA-(His)−ZnPP (10 μM) in potassium phosphate buffered solution (pH 7.0, 10 mM) at 25 °C. (B) UV−vis absorption spectra of ZnPPDME (10 μM) in toluene solution with EtOH (5 vol %) or 1-methylimidazole (5 mM) at 25 °C.

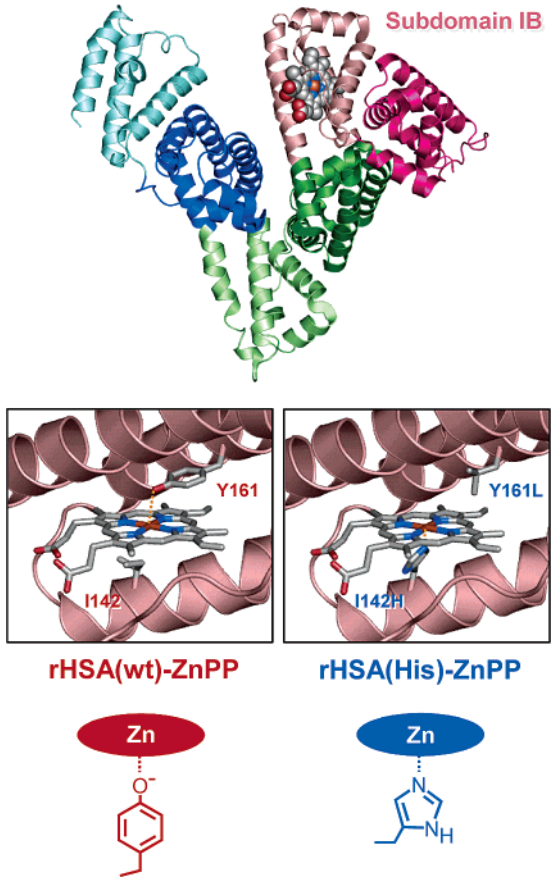


Figure 2. Structure models of rHSA complexed with ZnPP in subdomain IB (light red area).[9] In rHSA(wt), Tyr-161 axially coordinates to the central zinc ion of ZnPP. In rHSA(His), His-142 coordinates to the central zinc of ZnPP.

in the toluene solution. Since inhomogeneities of the porphyrin binding to rHSA would lead to a broadening of all the absorption spectra, the observed sharp and intense absorption bands of rHSA−ZnPP are consistent with a single binding site for the porphyrin chromophore. In fact, prosthetic heme groups in natural hemoproteins mostly show sharp absorption spectra. The fluorescence spectroscopy of rHSA(wt)−ZnPP and rHSA(His)−ZnPP also gave the same pattern of ZnPPDME with EtOH and 1-MIm, respectively (Figure S1). All these results imply that ZnPP is bound in subdomain IB of rHSA(wt) by the axial coordination of Tyr-161 and in rHSA(His) by the His-142 coordination (Figure 2). It is noteworthy that ZnPP can be monomolecularly dissolved in water at pH 5−8 by complexing it with rHSA at concentrations up to 3 mM.

The transient absorption spectrum of the degassed solution of rHSA(wt)−ZnPP upon laser flash photolysis at 532 nm showed the typical triplet−triplet (T−T) absorption of ZnPP (λ_{max} = 457, 673, 746 nm) (Figure S2).[10] The time course of the absorbance decay followed a single exponential kinetics with a triplet lifetime (τ_T) of 11 ms (Figure S3), which is considerably longer than that of $ZnTMPyP^{4+}$ (0.81 ms).[2a] The decay profile of the photoexcited ZnPP triplet is independent of the rHSA-(wt)−ZnPP concentration. In general, the triplet kinetics of a metalloporphyrin solution at a comparable concentration is dominated by the bimolecular exchange process.[11] In our case, (i) the ZnPP chromophore is held in a fixed position within the heme pocket of rHSA with a proximal base coordination, and (ii) the surface potential of rHSA is always negative at the neutral pH region because of its low isoelectric point of 4.8. Thus, the shielding of ZnPP by the negatively charged protein matrix appears to prevent a direct encounter between the excited triplets, and thereby inhibits a bimolecular T−T annihilation process. The same behavior was observed in Zn-substituted myoglobin.[12,13] The τ_T of rHSA(His)−ZnPP (2.5 ms) was one-fourth of the value observed for rHSA(wt)−ZnPP; the Tyr-161 coordination is clearly better at promoting the triplet state compared to the proximal His-142.

Photoinduced Electron Transfer to MV^{2+}. We investigated the photoinduced electron transfer reaction from rHSA−ZnPP to the water-soluble cationic acceptor, MV^{2+}. The addition of MV^{2+} resulted in a significant decrease in the triplet lifetime of rHSA(wt)−ZnPP. The transient absorption spectrum at 100 ns after the laser flash photolysis showed the same pattern as that without MV^{2+} (Figure 3A), while the spectrum after 200

(9) The picture was produced on the basis of crystal structure coordinate of the rHSA(wt)−hemin (code: 1O9X, ref 7) using PyMOL. DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientific: San Carlos, CA, 2006.
(10) Aono, S.; Nemoto, S.; Okura, I. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 591−593.
(11) Pekkarinen, L.; Linschitz, H. *J. Am. Chem. Soc.* **1960**, *82*, 2407−2411.
(12) Tsukahara, K.; Asami, S.; Okada, M.; Sakurai, T. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 421−431.
(13) Zemel, H.; Hoffman, B. M. *J. Am. Chem. Soc.* **1981**, *103*, 1192−1201.

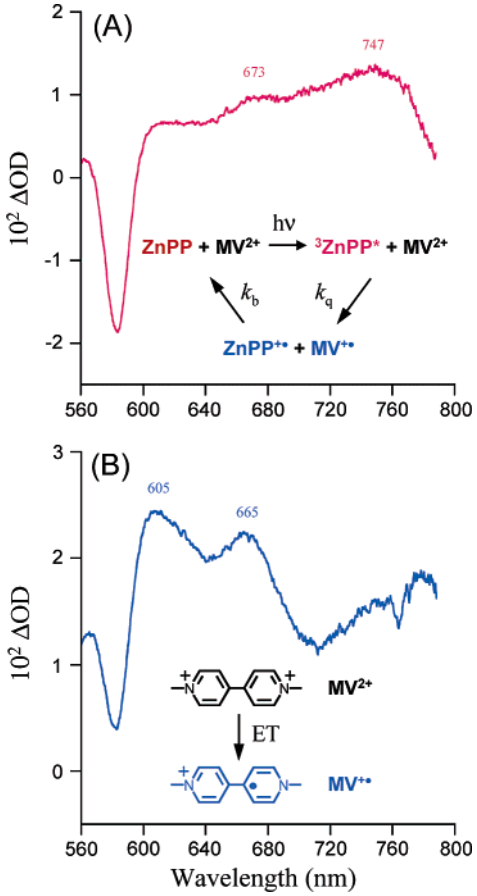


Figure 3. Transient absorption spectra of rHSA(wt)−ZnPP (10 μM) with MV^{2+} (1 mM) in phosphate buffered solution (pH 7.0, 10 mM) after the laser flash photolysis (λ = 532 nm) under argon atmosphere at 25 °C. (A) Spectrum after 100 ns, and (B) spectrum after 200 μs.

μs exhibited a very different shape with new peaks at 605 and 665 nm (Figure 3B). The intensity of these bands increased with the bleaching of the T−T absorption of ^{3}ZnPP*, indicating the oxidative quenching of rHSA(wt)−^{3}ZnPP* by MV^{2+}. The peak at 605 nm is characteristic of the one-electron-reduced MV^{2+} ($MV^{+\bullet}$), and the peak at 665 nm originates from the π-cation radicals of ZnPP ($ZnPP^{+\bullet}$).[1a,2,10,14] Concentration of MV^{2+} as high as 2 mM showed no influence on either the grand state absorption spectrum or the lifetime of the lowest-energy excited singlet state of rHSA(wt)−ZnPP (τ_S = 2.44 ns).[15] We conclude that there is no singlet reaction and that any observed photochemistry is attributed to the triplet pathway.

The triplet lifetime of rHSA(wt)−^{3}ZnPP* decreased linearly with the increasing MV^{2+} concentration to yield the Stern−Volmer constant (K_{SV}) of 1.5 × 10^5 M^{-1} (Figure 4).[16] The quenching rate constant (k_q) was determined to be 1.4 × 10^7 M^{-1} s^{-1}, indicating that it is almost diffusion controlled (Table 1). The charge-separated radical ions were reasonably long-lived and decayed via second-order kinetics. The rate constant of the thermal backward electron transfer (k_b) was 4.7 × 10^8 M^{-1} s^{-1}.[12] This can also be attributed to diffusional recombination. Similar kinetic parameters of rHSA(His)−ZnPP with MV^{2+}

(14) Liang, N.; Mauk, A. G.; Pielak, G. J.; Johnson, J. A.; Smith, M.; Hoffman, B. M. *Science* **1988**, *240*, 311−313.
(15) The τ_S of rHSA(wt)−ZnPP was 2.44 ns in the absence of MV^{2+} and 2.46 ns in the presence of 2 mM MV^{2+}.
(16) $\tau_T^o/\tau_T = K_{SV}[MV^{2+}]$ (τ_T^o and τ_T represent triplet lifetimes in the absence and presence of MV^{2+}).

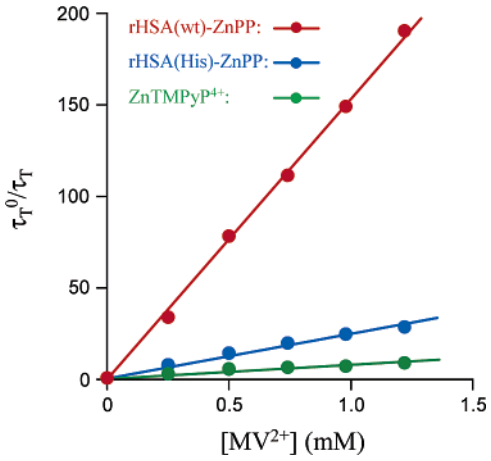


Figure 4. Stern−Volmer plots of triplet state quenching for rHSA−ZnPP (10 μM) with MV^{2+} in phosphate buffered solution (pH 7.0, 10 mM) at 25 °C.

Table 1. Rate Constants for Photoinduced Electron Transfer Reaction of rHSA−ZnPP to MV^{2+} in Phosphate Buffered Solution (pH 7.0, 10 mM) at 25 °C

Zn−porphyrin	k_q (M^{-1} s^{-1})	k_b (M^{-1} s^{-1})
rHSA(wt)−ZnPP	1.4 × 10^7	4.7 × 10^8
rHSA(His)−ZnPP	1.1 × 10^7	6.8 × 10^8
Zn−myoglobin[a]	2.7 × 10^7	4.0 × 10^7
$ZnTMPyP^{4+}$	9.3 × 10^6	9.8 × 10^8
	1.8 × 10^7 [b]	3.7 × 10^8 [b]
	2.0 × 10^6 [c]	

[a] From refs 10 and 12. [b] Ionic strength of the media was high (0.05); ref 2a. [c] From ref 1a.

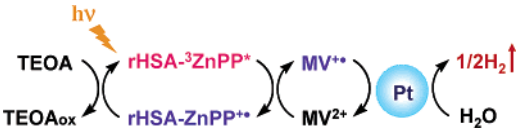
imply the same mechanism of the quenching. Both the rHSA(wt)−ZnPP and rHSA(His)−ZnPP showed a higher k_q and lower k_b relative to $ZnTMPyP^{4+}$ under the same conditions (Table 1).

The probability of quenching the triplet state by a particular concentration of MV^{2+} (ϕ_q) is expressed as $k_q[MV^{2+}]/((\tau_T^o)^{-1} + k_q[MV^{2+}])$.[2a] Even in the case of 1 mM MV^{2+}, ϕ_q is nearly 1.0; the photoexcited rHSA(wt)−^{3}ZnPP* was almost completely quenched by MV^{2+}.

Upon steady-state light irradiation (550 ± 2 nm) of the phosphate buffered solution (pH 5.4, 50 mM) of rHSA(wt)−ZnPP (10 μM) in the presence of MV^{2+} (2 mM) and TEOA (0.19 M), the color of the solution turned blue due to the accumulation of the $MV^{+\bullet}$ (λ_{max} = 605 nm). The reaction mechanism involves the electron transfer from rHSA(wt)−^{3}ZnPP* to MV^{2+}, followed by the reduction of rHSA(wt)−$ZnPP^{+\bullet}$ by TEOA. The intensity of the Soret band did not diminish, indicating no photodegradation of the ZnPP sensitizer. This is in rather sharp contrast to the fact that ZnPPDME in toluene (10 μM) was bleached with a half-life of ca. 10 min under the same conditions. The solution of rHSA(wt)−ZnPP absorbed 8.9 × 10^{16} photons for the initial 60 s, and 5.3 × 10^{15} molecules of $MV^{+\bullet}$ were produced; the overall quantum efficiency was calculated to be 5.9% [rHSA(His)−ZnPP = 5.6%].

Hydrogen Evolution from Water. Reduced MV^{2+} is well-known to drive the reduction of water to H_2 in the presence of a colloidal Pt catalyst. It should be possible to exploit the rHSA−ZnPP sensitizer for the light-induced H_2 evolution from water (Scheme 1). Colloidal PVA−Pt (20 μM) was then coupled

Scheme 1. Diagram of Photoinduced Reduction of Water Using rHSA−ZnPP as Sensitizer in Conjunction of MV^{2+} as Electron Relay, TEOA as Sacrificial Reagent, and Colloidal PVA−Pt as Catalyst



with the above phosphate buffered solution (pH 5.4, 50 mM) of the mixture consisting of rHSA(wt)−ZnPP (10 μM), MV^{2+} (2 mM), and TEOA (0.19 M). The coexistence of PVA−Pt and TEOA did not cause any λ_{max} shift in the UV−vis absorption of rHSA−ZnP, suggesting that the protein structure and the five-coordinate complex form of ZnPP in rHSA were unaltered. Upon exposure of this solution to a 450 W xenon arc lamp with an HA30 heat absorption filter (330−700 nm), the photogeneration of H_2 was observed without a decrease in activity during the experimental period (Figure 5). The volume of H_2 produced from a 3.5 mL solution of the complex following 6 h illumination with 57 turnovers was 0.044 mL. The rHSA(His)−ZnPP also exhibited an identical activity for the photosensitized reduction of water to H_2. We could not detect H_2 at the same experiments performed without the PVA−Pt catalyst or without TEOA. It is quite remarkable that the efficiency of the H_2 evolution using our rHSA−ZnPP is 25−30% greater than that of the system using $ZnTMPyP^{4+}$.

Conclusion

It is likely that the intense visible solar spectrum and long triplet lifetimes of rHSA(wt)−ZnPP and rHSA(His)−ZnPP are responsible for the excellent photoreduction of water to H_2 by these complexes. This astonishing result for the blood protein sensitizer including natural protoporphyrin IX will serve as a trigger to create a new field in artificial photosynthetic chemistry and innovative solar energy conversion. Currently, rHSA(wt) is manufactured in an industrial scale,[17] which allows us to use this zinc−protein photosensitizer in practical applications.

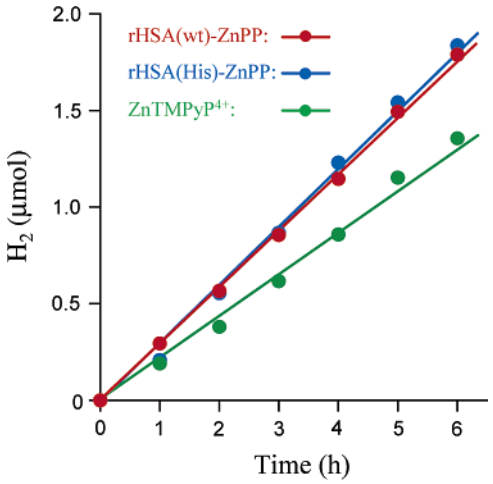


Figure 5. Time dependence of the H_2 production from water in the phosphate buffered solution (pH 5.4, 50 mM) of rHSA−ZnPP (10 μM), MV^{2+} (2 mM), TEOA (0.19 M), and colloidal PVA−Pt (20 μM) at 25 °C upon exposure to a 450 W xenon arc lamp with HA30 filter (330−700 nm).

Further research to optimize solute concentrations, catalyst preparation, and crystal structure analyses of the new rHSA−ZnPP series is now underway.

Acknowledgment. This work was supported by PRESTO, JST, Grant-in-Aid for Scientific Research from JSPS, and the Wellcome Trust (UK). The work at Imperial College London was partially carried out as the Japan−UK Research Cooperative Program (Joint Project) of JSPS.

Supporting Information Available: Fluorescence spectra, transient absorption spectra, and triplet absorption decay of rHSA(wt)−ZnPP and/or rHSA(His)−ZnPP. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0656806

(17) Kobayashi, K. *Biologicals* **2006**, *34*, 55−59.