Supplementary Information

Photocatalytic Hydrogen Generation Using Protein-Coated Photosensitizer with Anionic Patches and Monocationic Electron Mediator

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Instruments. ^{1}H-NMR spectra were collected using a Bruker DPX-400 (400 MHz) NMR spectrometer. The ^{1}H NMR chemical shifts are reported in ppm relative to the residual solvent resonances. The UV-visible experiments were conducted using a Shimadzu UV-3150 double beam spectrometer. The continuous Xe lamp irradiation was carried out by using an USHIO Optical Modulex SX-UI501XQ (500 W). The gas chromatographic (GC) experiments were performed using a Shimadzu GC-2014 gas chromatograph with a TCD detector. Laser flash photolysis was carried out with a laser flash photolysis system constructed by Unisoku, Co., Ltd. (Osaka, Japan). A Xe Arc lamp was employed as a source of the probe light to follow spectral changes. For laser flash photolysis, a sample was excited with 5-ns pulses (532 nm) from a Q-switched Nd:YAG laser (Surelite I, Continnum).

Materials. All reagents and chemicals were obtained from commercial sources and used as received unless otherwise noted. Myoglobin (Mb) from horse heart was purchased from Sigma (100684-32-0) and purified by column chromatography through CM-52 (Whatman) and Sephadex G-25 (GE healthcare) columns. PVP-coated Pt colloid was purchased from Tanaka Kikinzoku, Co., Ltd. (Tokyo, Japan). Syntheses of zinc protoporphyrin IX derivatives (**1** and **2**) and preparation of the reconstituted myoglobin, rMb(**1**), were described in the previous report.[1] 1-Methyl-4,4'-bipyridinium (**MMBP$^+$**) hydrochloride was prepared as described below.

Synthesis of 1-methyl-4,4'-bipyridinium (MMBP$^+$) hydrochloride. A solution of 4,4'-dipyridyl (1.20 g, 7.7 mmol) and methyl iodide (0.60 mL, 8.4 mmol) in dichloromethane (20 mL) was refluxed for 2 h. After the mixture was cooled to room temperature, the precipitate was filtered and washed with ethyl acetate. The crude product was recrystallized from methanol. The obtained yellow solid was dissolved in water and passed through an anion exchanger column (Dowex l-X8). The fractions containing the product (checked by TLC) were collected, and the solvent was removed under reduced pressure. The residue was washed by ethanol to give the desired compound as a white solid (1.03 g, 5.0 mmol, 65 %). ^{1}H NMR (D_2O, 400 MHz): δ = 8.76 (2H, d), 8.63 (2H, d), 8.24 (2H, d), 7.76 (2H, d), 4.29 (3H, s).

UV-vis measurements during continuous irradiation of rMb(1). The continuous irradiations of rMb(**1**) were carried out using a 500 W Xe lamp with an absorption filter (< 380 nm) to eliminate UV-region light. A solution of rMb (**1**) (14 μM) in potassium phosphate buffer (10 mM, pH = 7.0) including **MMBP**$^+$ or **DMBP**$^{2+}$ (2.0 mM) and TEOA (20 mM) was gently stirred in a quartz cuvette (L = 10 mm) under a N_2 atmosphere at 25 °C. The solution was irradiated with 5.0×10^4 lux, keeping the distance of 10 cm between the sample cuvette and the light source. In the period of the defined time, the sample solution was subjected to the UV-vis measurements.

Laser flash photolysis (Observation of decay of triplet state). The decay of the triplet state in rMbs (10 μM) in potassium phosphate buffer (10 mM, pH = 7.0) containing **MMBP**$^+$ or **DMBP**$^{2+}$ was observed by following the absorbance change at 460 nm after laser flash under a N_2 atmosphere at 25 °C. In the case of rMb(**1**), the absorbance changes were analyzed by double-exponential rate law to yield two apparent rate constants. The rate constants for the fast phase were plotted against [**MMBP**$^+$] or [**DMBP**$^{2+}$] and the saturated kinetic profile was analyzed by the method described in the previous report. The rate at the slow phase was constant with all the concentrations of the bipyridinium mediator. For rMb(**2**), the absorbance changes at 460 nm were analyzed by single-exponential rate law. The apparent rate constants with linear dependence on the concentrations of the bipyridinium mediator were analyzed by pseudo-first order kinetics.

Laser flash photolysis (Observation of decay of Zn cation radical). The generation and the decay of zinc π-cation radical in the reconstituted myoglobin, rMb(**1**) were observed by following the absorbance changes at 680 nm. The concentrations of rMb(**1**) and a bipyridinium mediator are 50 μM and 500 μM, respectively.

H_2 generation mediated by reconstituted myoglobins. A degassed mixture of a rMb (10 μM), a pyridinium mediator (1.0 mM), TEOA (20 mM) and a colloidal Pt-PVP (20 μM) dissolved in phosphate buffer (10 mM, pH = 7.0, 3.0 mL) at a quartz cuvette (L = 10 mm) was irradiated in the same manner as described above. The dead-space gas (100 μL) was sampled by a gastight syringe every 30 min without air contact, which was applied to GC equipped with an activated charcoal column and a TCD detector to determine the amount of generated hydrogen. Hydrogen was detected with the retention time of 0.44 min.

References

1 (a) T. Hayashi and Y. Hisaeda, *Acc. Chem. Res*. 2002, **35**, 35; (b) Y. Hitomi, T. Hayashi, K. Wada, T. Mizutani, Y. Hisaeda and H. Ogoshi, *Angew. Chem. Int. Ed.*, 2001, **40**, 1098; (c) T. Hayashi, T. Ando, T. Matsuda, H. Yonemura, S. Yamada and Y. Hisaeda, *J. Inorg. Biochem.*, 2000, **82**, 133; (d) T. Hayashi, T. Takimura, H. Ogoshi, *J. Am. Chem. Soc*., 1995, **117**, 11606.

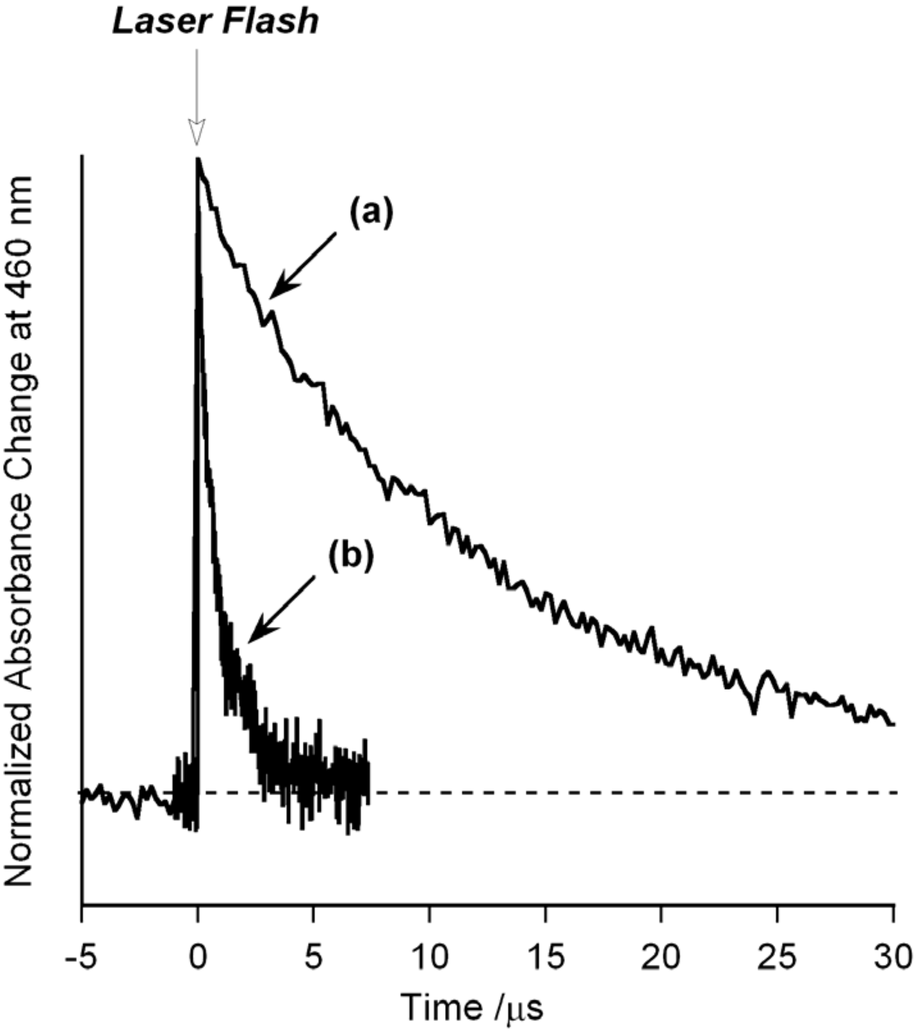


Fig. S1. Time-course of the absorbance at 460 nm after laser flash irradiation (Nd-YAG, 532 nm, 5 ns-pulse) for the triplet state of rMb(**1**) in the absence and presence of **DMBP**$^+$; 10 mM KPi, pH = 7.0, 25ºC; (a) in the absence of **DMBP**$^+$; (b) in the presence of **DMBP**$^+$ (100 μM).

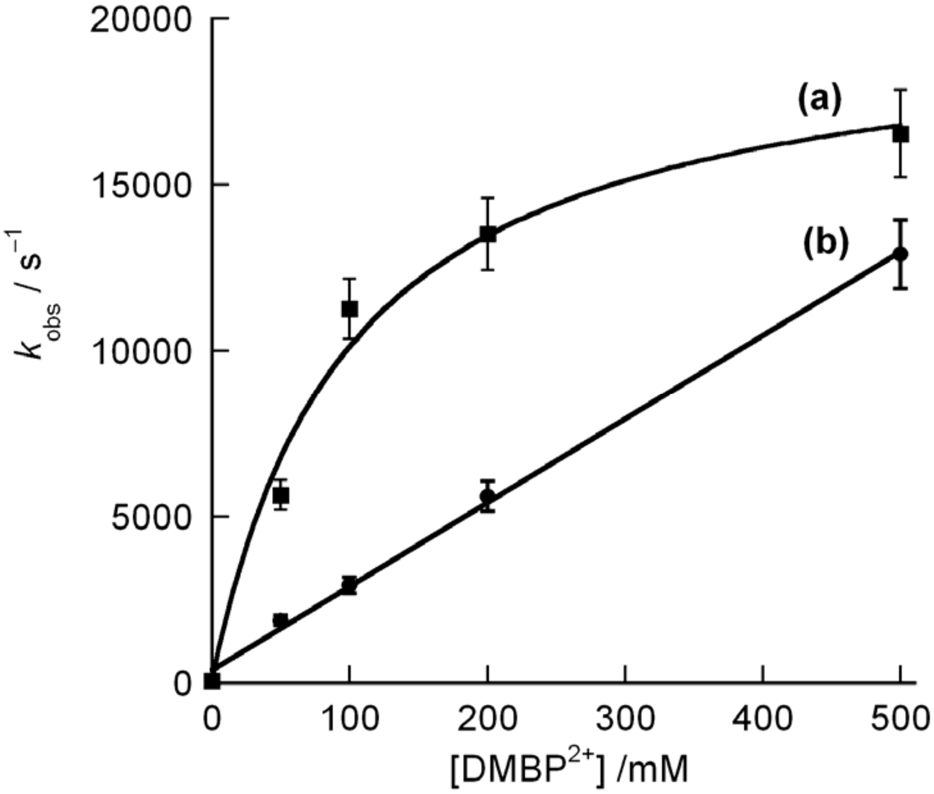


Fig. S2. Dependence of decay rates for the triplet state of rMb(**1**) after laser flash irradiation (Nd-YAG, 532 nm, 5 ns-pulse); 10 mM KPi, pH = 7.0, 25 ºC; (a) rMb(**1**) + **DMBP**$^+$ (fast phase); (b) rMb(**2**) + **DMBP**$^+$ (without the anion domain).

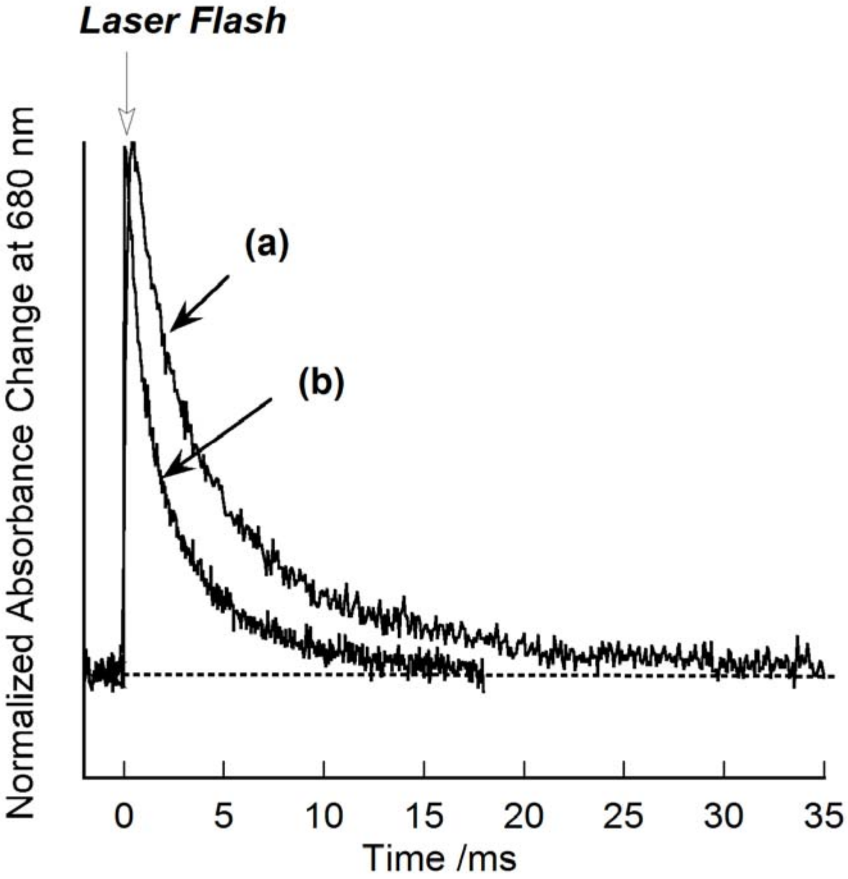
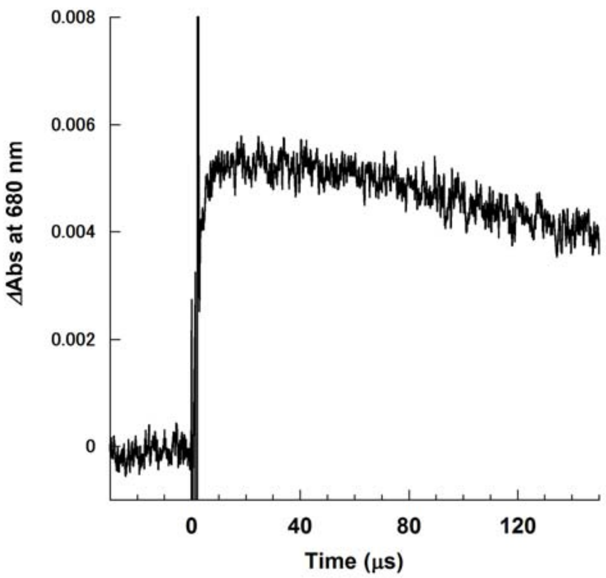


Fig. S3. Time-course of the absorbance at 680 nm (*normalized*) after laser flash irradiation (Nd-YAG, 532 nm, 5 ns-pulse) for the Zn radical cation; 10 mM KPi, pH = 7.0, 25ºC; (a) **MMBP**$^{\cdot}$ (500 μM); (b) **DMBP**$^{\cdot+}$ (500 μM).

(a) MMBP^{+}



(b) DMBP^{2+}

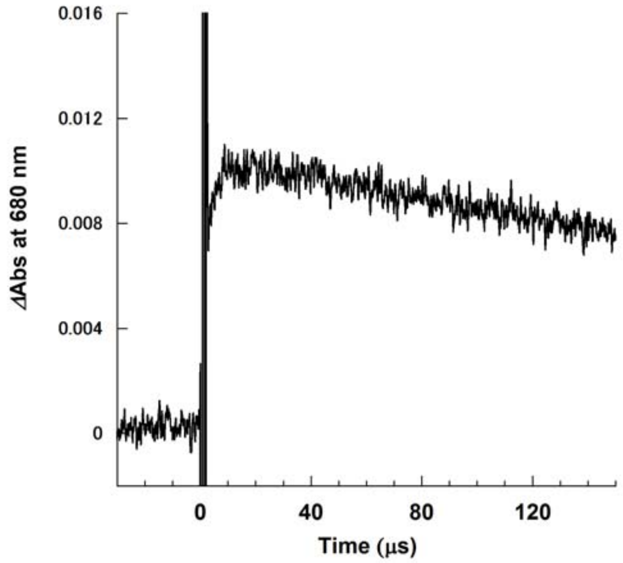


Fig. S3. Time-course of the absorbance at 680 nm (*observed at the initial stage*) after laser flash irradiation (Nd-YAG, 532 nm, 5 ns-pulse) for the Zn radical cation; 10 mM KPi, pH = 7.0, 25ºC; (a) **MMBP**$^{\cdot}$ (500 μM); (b) **DMBP**$^{\cdot+}$ (500 μM).