Photocatalytic hydrogen generation using a protein-coated photosensitizer with anionic patches and a monocationic electron mediator†

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A reconstituted myoglobin with a synthetic cofactor having anionic binding sites effectively works as a photocatalyst for hydrogen generation in the presence of monomethylated bipyridinium.

Hydrogen (H_2) generation mediated by a photosensitizer (photocatalyst) has been paid significant attention as a candidate for a clean energy source without the emission of CO_2.[1] Especially, the production of H_2 in water is attractive because water acts as a "proton pool" ($2H^+ + 2e^- \rightarrow H_2$). Although naturally-occurring hydrogenases could be used for the H_2 production from water,[2] artificially constructed systems, ideally, the splitting of water using light energy, should also be developed from the aspect of a tailor-made designed system. The majority of artificial systems for H_2 generation thus reported are composed of a π-conjugated dye photocatalyst, an electron mediator, a metal catalyst for H_2 generation and a sacrificial reagent.[3–5] One of the most popular systems is the combination of a porphyrin derivative (**P**), methyl viologen (1,1′-dimethyl-4,4′-bipyridinium, **DMBP²⁺**), Pt-colloid and a reducing reagent such as tertiary amine (**TA**). The system is schematically described in Scheme 1.[6]

However, the following mechanisms should be developed for the assembly of components to construct a system that effectively generates H_2: (i) dispersion of self-aggregative photosensitizer molecules, (ii) effective photo-induced electron transfer (PET) from excited photocatalyst to electron mediator, and (iii) suppression of back electron transfer (BET) in the charge separated state (*e.g.*, **P•⁺** and **DMBP•⁺** in Scheme 1). In this context, the reconstituted myoglobin with a synthetic cofactor **1** (rMb(**1**))[7] has emerged as a photocatalytic system for H_2 generation (Fig. 1).[8]

Zinc porphyrin installed in **1** is one of the versatile photosensitizers. When the porphyrin moiety is masked by the protein matrix of myoglobin, the self-aggregation of the hydrophobic porphyrin will be suppressed. The anionic domain introduced at the terminal of the heme propionates would effectively bind a cationic electron mediator to attain
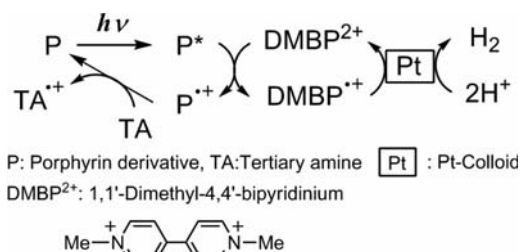
Scheme 1 Typical mechanism of H_2 generation by a porphyrin derivative, tertiary amine, **DMBP²⁺** and Pt-colloid.

the smooth PET. Furthermore, we examined a monocationic bipyridinium, 1-methyl-4,4′-bipyridinium (**MMBP⁺**), as an electron mediator in place of **DMBP²⁺**. **MMBP⁺** is supposed
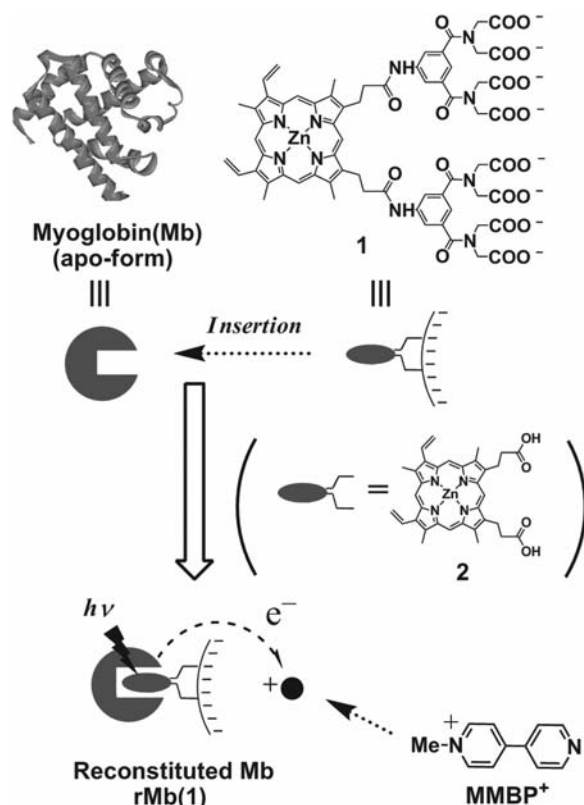

Fig. 1 Reconstitution of myoglobin (Mb) with a synthetic cofactor having an anionic domain **1**.

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† Electronic supplementary information (ESI) available: Detailed experimental procedures. See DOI: 10.1039/b803491e
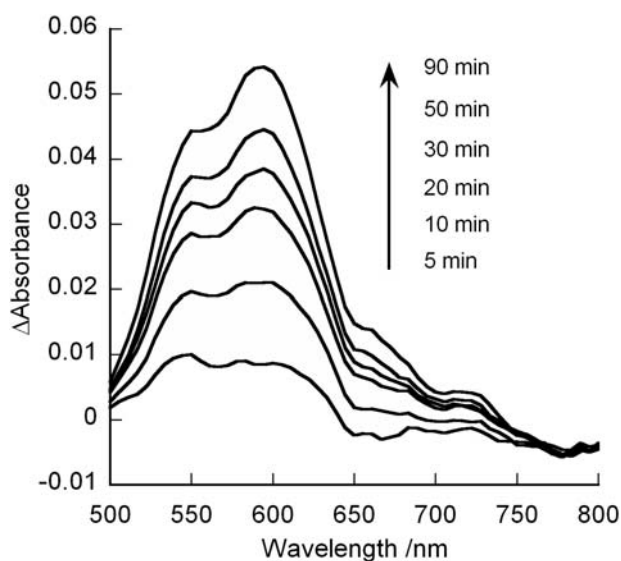
Fig. 2 Formation of **MMBP•** by irradiation of rMb(**1**) using a Xe lamp (>380 nm) in the presence of **TEOA**; [rMb(**1**)] = 10 μM, [**MMBP$^+$**] = 2.0 mM, [**TEOA**] = 20 mM; 10 mM KPi, pH = 7.0, 25 °C.

Fig. 3 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 °C; (a) rMb(**1**) + **MMBP$^+$** (fast phase); (b) rMb(**2**) + **MMBP$^+$** (without the anion domain).

to effectively bind to the anionic domain, followed by the conversion into the electrostatically neutral species, **MMBP•**. The neutral species is expected to readily escape from the anionic domain. In contrast, the one-electron reduced form of **DMBP^{2+}**, **DMBP•$^+$**, would still remain in the vicinity of the anionic domain because of its cationic character even after reduction. This difference in the electrostatic states would be essential for suppressing the BET to the Zn cation radical generated after the PET.

Upon the continuous irradiation of rMb(**1**) by a Xe lamp (>380 nm) in the presence of **MMBP$^+$** and excess triethanolamine (**TEOA**) under anaerobic conditions, the formation of **MMBP•** was observed, featured by the increase in absorbance around 600 nm (Fig. 2). No spectral change was observed in the dark or under aerobic conditions, indicative of the PET from **1** in the protein to **MMBP$^+$**.

In order to investigate the kinetic properties of PET in rMb(**1**), laser flash experiments using a Nd-YAG laser (532 nm excitation, 5 nm pulse width) were carried out. After the photolysis of rMb(**1**) in the absence of **MMBP$^+$**, the decrease in the absorbance at 460 nm (triplet state of Zn porphyrin, **1***) was observed with a rate constant of 74 s^{-1}. Without the protein matrix, the decay of **1*** (10 μM) was accelerated (8.0 × 10^2 s^{-1}) due to the quenching by the collisions of the porphyrin moieties,[9] suggesting that the protein matrix acted as a mask for the porphyrin moiety. The decay of **1*** in rMb(**1**) upon irradiation displayed two-phase exponential kinetics. The rate of the fast phase was dependent on the concentrations of **MMBP$^+$** with Michaelis–Menten-type saturation kinetics (Fig. 3(a)). The slow phase was almost constant at the various concentrations of **MMBP$^+$** with a rate constant of 200 s^{-1}.[10] Therefore, the fast phase was identified as the PET process from **1*** in the protein to **MMBP$^+$** via the binding of **MMBP$^+$** to the anionic domain. One possibility of the events in the slow phase is a unimolecular process such as the conformational rearrangements of the mediator after the binding, based on the
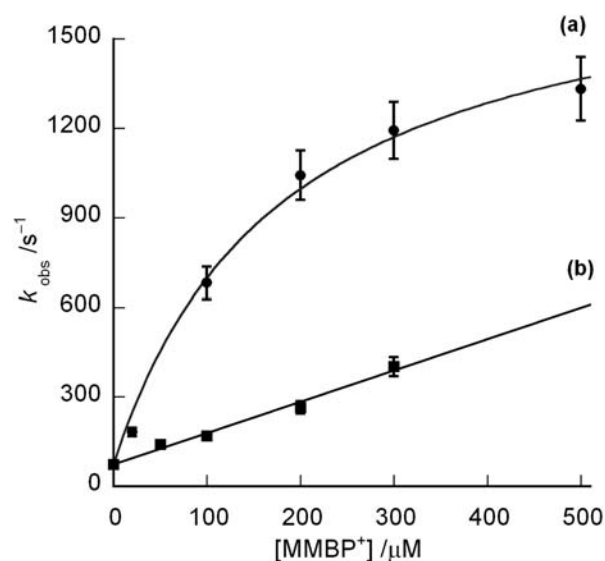
constant rate constant in various concentrations of **MMBP$^+$**. In contrast, the decay rate in rMb(**2**), the rMb having **2** without the anionic binding site, linearly depends on the concentrations of **MMBP$^+$** (Fig. 3(b)), indicating that the non-specific collisions of the protein with **MMBP$^+$** is the dominant process of PET in this case. The kinetic profile of PET from rMb(**1**) to **MMBP$^+$** was sharply different from that to **DMBP^{2+}**. The binding constants (K_a) and the rate of electron transfer (k_{et}) were calculated to be 5.4 × 10^3 M^{-1} and 1.8 × 10^3 s^{-1} for **MMBP$^+$**, respectively. For **DMBP^{2+}**, the corresponding values were determined to be 1.0 × 10^4 M^{-1} and 2.0 × 10^4 s^{-1}, respectively (See ESI† for the plot of the observed rate constant vs. [**DMBP^{2+}**]).[11] The difference of K_a is reflected by the charge number of the pyridiniums: 2+ for **DMBP^{2+}** or 1+ for **MMBP$^+$**. The small k_{et} in **MMBP$^+$** is explained by the negatively shifted redox potential of **MMBP$^+$**/**MMBP•** compared to **DMBP^{2+}**/**DMBP•$^+$**.[12] Therefore, at first glance, **MMBP$^+$** looks inferior as an electron mediator. However, we observed the slower decay of the Zn cation radical (680 nm) generated after the PET in the presence of **MMBP$^+$**; 6.9 × 10^2 s^{-1} for **MMBP$^+$** (500 μM) and 1.8 × 10^3 s^{-1} for **DMBP^{2+}** (500 μM), although **MMBP•** is expected to facilitate the BET to quench Zn cation radical because of its negative redox potential. This finding suggests that **MMBP•** more readily diffuses into the bulk than **DMBP•$^+$**, leading to the extension of the lifetime of the Zn cation radical. Therefore, **MMBP•** would effectively mediate the electron transfer between the triplet state of the Zn porphyrin and a metal catalyst for H$_2$ generation such as Pt-colloid with prevention of BET.

Next, we attempted the H$_2$ generation in KPi buffer (pH = 7.0) using rMb(**1**) (10 μM) in the presence of a pyridinum mediator (1 mM), PVP-coated Pt-colloid (20 μM) and excess triethanolamine (20 mM) (Scheme 2).

When we employed **MMBP$^+$** as the electron mediator (EM), the generation of H$_2$ was observed upon the irradiation
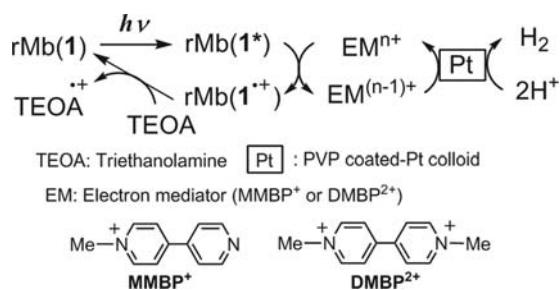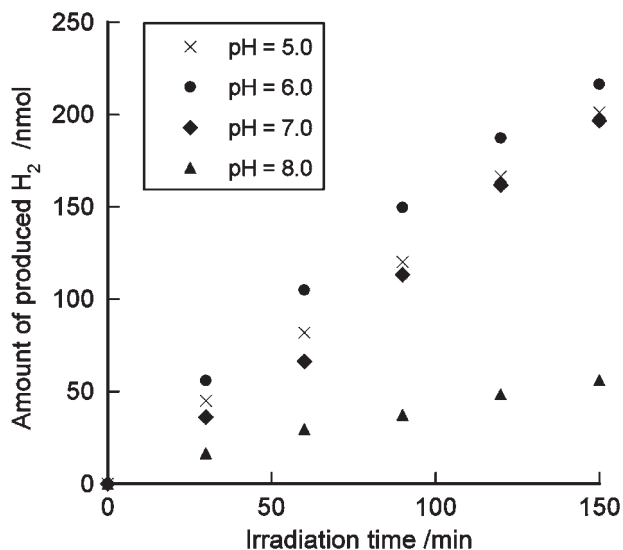
Scheme 2 System for H$_2$ generation using rMb(**1**).

Fig. 4 Amounts of H$_2$ generated by the catalytic system based on rMb(**1**); [rMb(**1**)] = 10 μM, [**MMBP$^+$**] = 1.0 mM, [TEOA] = 20 mM: 100 mM KPi, pH = 7.0, 25 °C.

by a 500 W Xe lamp (>380 nm). The amount of H$_2$ generated linearly increased over 150 min, but the production of H$_2$ was gradually suppressed after that time. The UV-Vis measurement of rMb(**1**) after the reaction showed that there was no photochemical degradation of the Zn porphyrin moiety. Therefore, the slowdown of the H$_2$ generation is probably due to the consumption of triethanolamine during the reaction. No H$_2$ generation was observed when even one of the components was missing or there was no irradiation. Therefore, the observed H$_2$ generation was based on the mechanism described by Scheme 2. Interestingly, the amount of generated H$_2$ was below the detection limit when **DMBP^{2+}** was employed in spite of a typical electron transfer. One of the reasons for this finding may be significant BET without electron transfer to Pt-colloid in the case of **DMBP$^{\bullet+}$**, whereas **MMBP$^\bullet$** is able to readily escape from the charged domain because of its electrostatically neutral characteristics. Furthermore, the negative redox potential of **MMBP$^\bullet$** would contribute to the favorable electron transfer to the Pt-colloid compared to **DMBP$^{\bullet+}$**.

The effect of the reaction media pH was also investigated (Fig. 4). The maximum efficiency was observed at pH = 6.0, although weakly acidic conditions around pH = 5.0 were employed in the previous research examples because the

production of H$_2$ depends on the concentrations of H$^+$.[3–6] This tendency may be explained by the protonation of the anionic domain at the surface of rMb(**1**), which interferes with the binding of the electron mediator.

In conclusion, we demonstrated H$_2$ generation by a myoglobin-coated photosensitizer with an anionic binding domain for a cationic electron mediator. It was found that employment of monomethylated bipyridinium, **MMBP$^+$**, effectively prevents the BET with a smooth electron transfer to a metal catalyst for the H$_2$ generation, although **DMBP^{2+}** is a popular electron mediator. This system will provide a key insight into constructing systems for H$_2$ generation while overcoming the problems caused by the structural characteristics of the photosensitizers and electron mediators.

Notes and references

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