Enhancement of Photoinduced Electron Transfer from Porphyrin to Methyl Viologen by Binding of an Antibody for Porphyrin

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Photoinduced electron transfer from porphyrins to electron acceptors was enhanced in the presence of a monoclonal antibody for porphyrin. A hydrogen evolution system was constructed by utilizing the antibody–porphyrin complex, methyl viologen, and colloidal Pt.

The photosynthetic system in vivo can convert solar energy to chemical energy with high efficiency.[1] The chromophores of the in vivo photosynthetic reaction center are fixed by the protein environment.[2] The distances from electron donors to electron acceptors and their relative position are noncovalently fixed under optimum conditions. Much effort has been directed toward mimicking the electron transfer in natural photosynthetic systems. There are many studies for electron-transfer system using covalently linked electron donor–acceptor molecules.[3] Recently, noncovalently assembled donor–acceptor arrays were constructed by utilizing hydrogen bonding,[4] metal coordination,[5] apoproteins,[6] and electrostatic interaction.[7] We have been preparing monoclonal antibodies for electron donors (porphyrins) as matrices for synthetic porphyrins[8a,8b] to construct a new energy-conversion system. The binding of antibodies to porphyrins could bring about a lengthening of excited state lifetime of porphyrins.[8c] In this report, we have prepared a monoclonal antibody for hapten **1** and constructed a hydrogen evolution system. Photoinduced electron transfer from porphyrin to an electron acceptor (methyl viologen, MV^{2+}) was enhanced in the presence of the antibody. We found that the binding of antibodies to porphyrins could lead to the generation of stable electron-transfer products.

An antibody was obtained by immunization of Balb/c mice with hapten **1** conjugeted to keyhole limpet hemocyanin. The antibody (2B6) was found to bind not only *meso*-tetrakis(4-carboxyphenyl)porphyrin (TCPP) but also TCPP zinc complex (Zn–TCPP) with the dissociation constants of 2.0×10^{-8} M (=mol dm^{-3}) and 2.1×10^{-8} M, respectively, estimated by enzyme-linked immunosorbent assay (ELISA). When antibody 2B6 was added to an aqueous solution of Zn–TCPP, the UV–vis spectra of Zn–TCPP showed a hyperchroism and peak shift toward a longer wavelength by 4 nm in the region of the Soret band. The circular dichroism (CD) spectra showed induced Cotton effect on Zn–TCPP, indicating that Zn–TCPP was incorporated into the chiral environment of the binding site of the antibody (Figure 1A) with a 1:1 binding mode.[8d] The florescence spectra of Zn–TCPP in the presence and absence of antibody 2B6 are shown in Figure 1B. The fluorescence intensity of Zn–TCPP increased by the addition of the antibody. The lifetime of its singlet exited state was lengthened from 1.7 to 2.1 ns.

The antibody was found to have the porphyrin binding site,

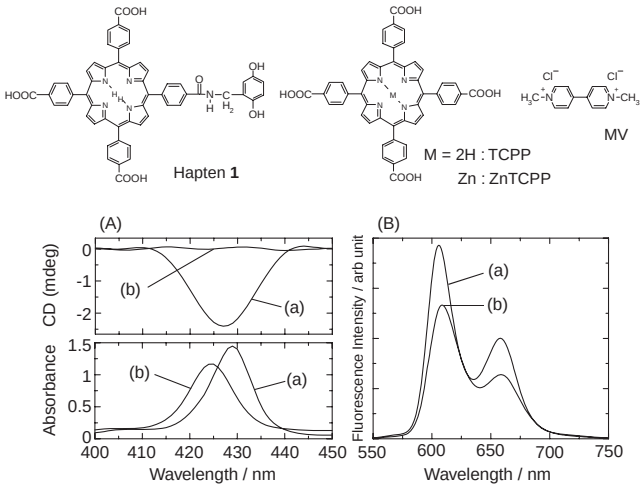

Figure 1. (A) CD (upper) and UV–vis (lower) spectra of Zn–TCPP in the presence (a) and absence (b) of antibody 2B6. (B) Fluorescence spectra of Zn–TCPP in the presence (a) and absence (b) of antibody 2B6. [Zn–TCPP] = 2.5×10^{-6} M, [antibody 2B6 (binding site)] = 5.0×10^{-6} M (a) and 0 M (b), and [tetrasodium ethylenediaminetetraacetate (EDTA-4Na)] = 1.25×10^{-3} M. Excitation wavelength was set at 424 nm.

however, there was no affinity for quinone or hydroquinone, which was a part of hapten molecule. We examined photochemical property of the antibody–porphyrin complex by using MV^{2+} as an electron acceptor instead of quinone derivatives. The fluorescence of Zn–TCPP decreased by the addition of MV^{2+} in the presence and absence of antibody 2B6 with a different quenching behavior. Figure 2 shows the Stern–Volmer plots for the quenching of the emission from the Soret band of Zn–TCPP by the addition of MV^{2+}. The ratio of fluorescence intensities of Zn–TCPP without MV^{2+} and that with MV^{2+} (I_0/I) increased linearly with an increase in the concentration of MV^{2+} in the absence of the antibody. With antibody 2B6, the values of I_0/I increased nonlinearly and were larger than that without antibodies. The dissociation of Zn–TCPP from the complex with the antibody by the addition of MV^{2+} was negligible because the CD intensity of Zn–TCPP was the same as that without MV^{2+}. Unexpected large fluorescence quenching of Zn–TCPP was observed in the complex with the antibody, suggesting that the number of MV^{2+} molecules, which can interact with Zn–TCPP, increased by the binding of the antibody to Zn–TCPP. The saturation behavior of fluorescence quenching of Zn–TCPP by MV^{2+} in the presence of antibody 2B6 as shown in Figure 2 indicated the limit of the number of MV^{2+} molecule approaching to Zn–TCPP in the binding pocket of the antibody. Although no

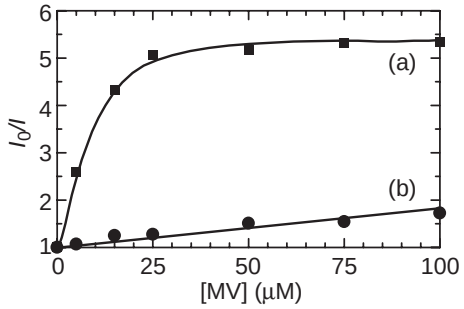

Figure 2. The Stern–Volmer plots for the quenching of the emission from the Soret band of Zn–TCPP by MV^{2+} (a) with and (b) without antibody 2B6. I_0 and I are the fluorescence intensities of Zn–TCPP at 605 nm in the absence and presence of MV^{2+}, respectively. [Zn–TCPP] $= 2.5 \times 10^{-7}$ M, [antibody 2B6 (binding site)] $= 5.0 \times 10^{-7}$ M (a) and 0 M (b), and [EDTA-4Na] $= 1.25 \times 10^{-3}$ M.

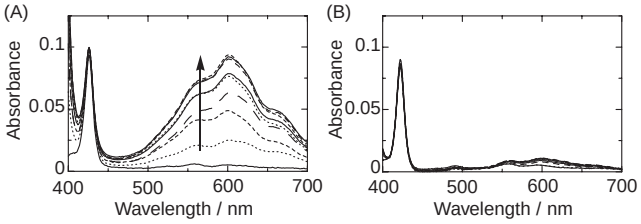

Figure 3. UV–vis spectral changes of the solutions of the mixture of Zn–TCPP and MV^{2+} by the irradiation of light in the presence (A) and absence (B) of antibody 2B6, recorded at 5 min intervals. [Zn–TCPP] $= 2.5 \times 10^{-7}$ M, [antibody 2B6 (binding site)] $= 5.0 \times 10^{-7}$ M (A) and 0 M (B), and [MV^{2+}] $= 1.0 \times 10^{-4}$ M in 0.1 M PBB (pH 9.0).

binding pocket of antibodies to MV^{2+} was designed, the antibody was indicated to have the affinity for MV^{2+} by electrostatic interactions. (See Supporting Information)

The continuous light irradiation to Zn–TCPP was performed in the presence of the antibody and MV^{2+}. The color of the solution turned to blue and a product with a maximum absorbance at 602 nm appeared (Figure 3A).[9] Methyl viologen cationic radical ($MV^{+\bullet}$) was obtained by the photoinduced electron transfer from porphyrin in the binding site of the antibody to MV^{2+}. $MV^{+\bullet}$ was generated even though the distance between Zn–TCPP and MV^{2+} was fixed by the antibody enough far to prevent them forming charge-transfer complexes. Antibody 2B6 was considered to hold MV^{2+} near its antigen binding site for Zn–TCPP, photoinduced electron transfer from Zn–TCPP to MV^{2+} could efficiently occur. The half-life of $MV^{+\bullet}$ was over than 15 min. Contradistinctively, no color changes were observed without antibodies (Figure 3B). The back electron transfer should occur in the absence of the antibody. The antibody was found to catalyze the electron transfer to give a stable $MV^{+\bullet}$.

We found that the stable $MV^{+\bullet}$ obtained in the antibody–porphyrin complex system could be utilized for producing chemical energy, hydrogen.[10] The antibody–porphyrin complex, MV^{2+}, and colloidal Pt were equipped for the construction of a hydrogen evolution system. The increase of the concentration of hydrogen was observed in the solution of the antibody–porphyrin complex.[11] The amount of produced hydrogen was estimated to be 1.1×10^{-5} M after one-hour irradiation. It was about 44 times as much as the amount of Zn–TCPP. On the other hand, no hydrogen production was observed in the absence of the antibody. It was suggested that $MV^{+\bullet}$, which was generated by the electron transfer from Zn–TCPP in the binding pocket of the antibody, delivered the electron to colloidal Pt and protons were reduced on the surface of colloidal Pt.

In conclusion, photoinduced electron transfer from Zn–TCPP to MV^{2+} was accelerated by the binding of antibody 2B6 to Zn–TCPP. The antibody was suggested to concentrate MV^{2+} around its binding pocket for porphyrin. The electron transfer could take place efficiently from porphyrin in the antibody binding site to MV^{2+} outside of the binding pocket. $MV^{+\bullet}$ generated by the irradiation of light to Zn–TCPP in the complex with antibody 2B6 was found to be stable with a long lifetime. A hydrogen evolution system was constructed by utilizing the antibody–porphyrin complex, MV^{2+}, and colloidal Pt.

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