Photoinduced Singlet Electron Transfer in a Complex Formed from Zinc Myoglobin and Methyl Viologen: Artificial Recognition by a Chemically Modified Porphyrin

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Received August 30, 1995

One major goal of molecular recognition processes in biological systems is to understand how the chemical reaction is regulated by protein binding sites. For example, it is well known that the specific interaction of the cytochrome *c*−cytochrome *c* peroxidase complex plays an important role in long-range electron transfer (ET).[1] However, the noncovalent model systems in which fast ET occurs from donor to acceptor via intermolecular interaction remain limited.[2] Here, we wish to report a nonmutagenic approach to the construction of the noncovalent ET model that is based on the complex of methyl viologen (**2**) and a myoglobin reconstituted with a modified zinc porphyrin (**1**·Zn) having an assembly of eight carboxylic acid groups as a specific interface. The present supramolecular system, designed to mimic the protein−protein complex of a reductoxidase, enables us to observe the fast singlet ET from zinc myoglobin to methyl viologen as a first example.

Our strategy for the construction of rMb(**1**·Zn)−**2** pairing is shown in Scheme 1.[3] Eight carboxylates attached to 6- and 7-propionates of mesoporphyrin IX were designed as an artificial recognition site for the cationic acceptor.[4] Free base **1** was prepared by the coupling of mesoporphyrin and 5-aminoisophthalate condensed with two aspartic acids. The synthetic zinc porphyrin **1**·Zn was easily inserted into horse heart apomyoglobin by a routine method.[5−7] The characteristic visible absorption spectrum of the reconstituted protein was similar to that of reference protein reconstituted with mesoporphyrin zinc complex **3**·Zn.

Addition of **2** to the solution of rMb(**1**·Zn) brought about no changes in the visible absorption spectrum. In contrast, fluorescence quenching in rMb(**1**·Zn) clearly was observed upon addition of **2** in 10 mM phosphate buffer, pH 7.0, while reference reconstituted myoglobin, rMb(**3**·Zn), showed no fluorescence quenching in the presence of **2** (0.01 M). Stern−Volmer plots obtained from fluorescence titration studies indicate that there is efficient quenching of zinc porphyrin fluorescence emission at 580- and 635-nm wavelengths in a low concentration of **2** ($< 6 \times 10^{-3}$ M) (Figure 1), since the static quenching occurs due to the photoinduced ET from rMb(**1**·Zn) to **2**. Figure 1 further reveals that the efficiency of quenching increases with increasing pH of the solution. The observed pH dependence is evidently due to the dissociation of four aspartic acids at the interface. Furthermore, at high ionic strength (pH 7.0, 100 mM phosphate buffer), the quenching efficiency is obviously smaller than that at low ionic strength, as shown in Figure 1. It is noted that the complex formation of rMb(**1**·Zn) and **2** is effective in the quenching of fluorescence emission of the zinc porphyrin in protein.

The fluorescence decay of rMb(**1**·Zn) was expressed by a monoexponential curve with a lifetime of 2.0 ± 0.1 ns, whereas addition of **2** brought about a biphasic decay curve expressed by two lifetimes: $\tau_L = 2.0 \pm 0.1$ ns (50%) and $\tau_S = 0.38 \pm 0.1$ ns (50%) at $[\mathbf{2}] = 1.1 \times 10^{-3}$ M. The longer- and shorter-lived components of the decay curve are assignable to free rMb(**1**·Zn) and the rMb(**1**·Zn)−**2** complex, respectively, and the magnitude of each component depends on the concentration of **2**. The derived rate constant for ET is $k_{et}^S = 2.1 \times 10^9$ s^{-1}.[8] Transient absorption spectra of rMb(**1**·Zn) in the presence of **2**

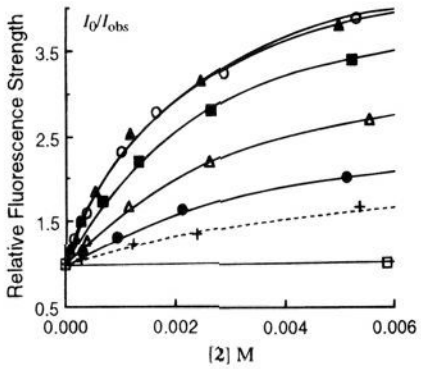
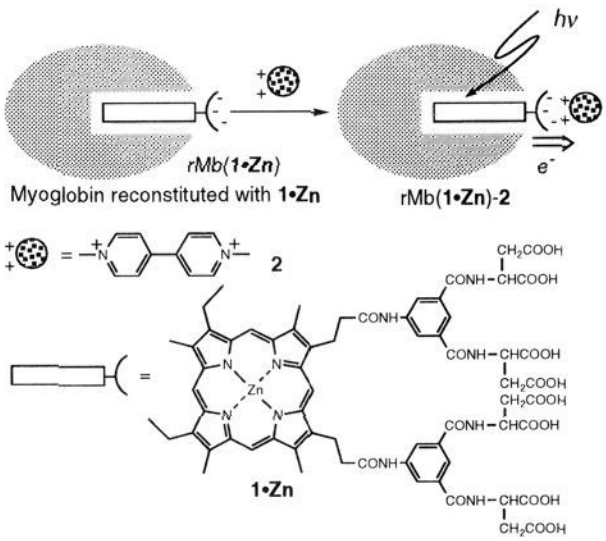


Figure 1. Stern−Volmer plots for rMb(**1**·Zn) quenched by **2** with various pH values at 25 °C. pH = 5.76 (●), 6.10 (△), 6.41 (■), 7.08 (▲), 8.32 (○) in 10 mM phosphate buffer. The dashed line corresponds to the data obtained in 100 mM phosphate buffer, pH 7.0. The open square corresponds to the plot for rMb(**3**·Zn) in pH 7.0, 10 mM phosphate buffer.

Scheme 1



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(3) Throughout this paper, myoglobins reconstituted with **1**·Zn and **3**·Zn are abbreviated as rMb(**1**·Zn) and rMb(**3**·Zn), respectively.

(4) 5-[(*tert*-Butoxycarbonyl)amino]isophthaloyldiaspartic acid as a precursor interacts with **2** in pH 7.0 phosphate buffer solution. The affinity constant was determined to be (3.6 ± 0.4) × 10^2 M^{-1} by ^{1}H NMR measurement.

(5) See supporting information for preparation details.

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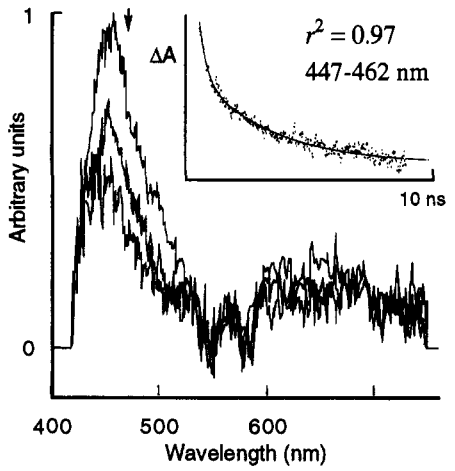

Figure 2. Transient absorption spectra for rMb(**1**·Zn) in the presence of **2** at 0.30−0.35, 1.31−1.36, and 6.45−6.50 ns after a 532-nm, 50-ps laser flash in pH 7.0, 10 mM phosphate buffer at 25 °C. Inset: Typical fitted decay trace for data obtained at 447−462 nm. The experimental data points are represented by dots; the solid curve is the fit to the modified Mataga's equation[11] with $\tau_1 = 0.31 \pm 0.1$ ns and $\tau_2 = 3.3 \pm 0.2$ ns.

give us direct evidence of ET from rMb(**1**·Zn) to **2**. Figure 2 shows the spectral changes monitored by streak camera at 0.30−0.35, 1.31−1.36, and 6.45−6.50 ns after a 50-ps, 532-nm laser flash for rMb(**1**·Zn)−**2** in pH 7.0 phosphate buffer.[9] A strong absorption feature centered at 455 nm, which decays within 6 ns, and a broad absorption between 600 and 750 nm were observed. These absorptions are assigned to the overlap of excited singlet and triplet states and cation radical species of zinc porphyrin in protein.[10] The time dependence of this change, centered at 455 nm, is analyzed by a modified version of Mataga's equation:[11] $\tau_1 = 0.31 \pm 0.1$ ns and $\tau_2 = 3.3 \pm 0.2$ ns, where $\tau_1 = 1/(k_{et} + k_{isc} + k_{ic} + k_f)$ and $\tau_2 = 1/k_{cr}$ (inset of Figure 2). The former value, τ_1, is consistent with τ_S determined by fluorescence lifetime measurement, and this agreement demonstrates the reliability of experimental data. The latter value, τ_2, indicates the degree of charge recombination of zinc cation radical with a rate constant of $k_{cr} = 3.3 \times 10^8$ s^{-1}. In contrast, the transient absorption spectra for rMb(**3**·Zn) in the presence of **2** show no clear decay of absorption in same time range.[12]

In conclusion, the present approach by the reconstituted zinc myoglobin may give a deeper insight into the mechanism of long-range ET and allow us to understand the ET regulated by the enzyme−enzyme or enzyme−coenzyme complex formation.

Acknowledgment. We are grateful to Mr. T. Ito and Mr. H. Saito, Hamamatsu Photonics K. K., for the measurement of transient absorption spectra. This work was supported by a Grant-in-Aid for Specially Promoted Research (No. 04101003) from the Ministry of Education, Science, and Culture, Japan.

Supporting Information Available: Preparation of zinc porphyrin and reconstituted myoglobin, procedure for photophysical measurements, and transient absorption spectra (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(8) The ET rate constant was derived from $k_{et}^S = 1/\tau_S - 1/\tau_L$.

(9) Description of the apparatus is described in the following: Ito, T.; Hiramatsu, M.; Tsuchiya, Y. *Rev. Sci. Instrum.* **1991**, *62*, 1415. See supporting information.

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(11) The difference absorption at 447−462 nm is represented as follows: $\Delta A_{447-462} = \{\Delta\epsilon^{*1}{}_{P\text{-}MV} - \Delta\epsilon^{*3}{}_{P\text{-}MV}{}^{\bullet}\tau_1 k_{isc}\}[^{*1}P\text{-}MV]_0\{\exp(-t/\tau_1)\} + \{\Delta\epsilon^{+}{}_{P\text{-}MV}{}^{-\bullet}\tau_1 k_{et}\}[^{*1}P\text{-}MV]_0[\{\tau_2/(\tau_1 - \tau_2)\}\{\exp(-t/\tau_1) - \exp(-t/\tau_2)\} + \{\Delta\epsilon^{*3}{}_{P\text{-}MV}{}^{\bullet}\tau_1 k_{isc}\}[^{*1}P\text{-}MV]_0$, where $^{*1}P$, $^{*3}P$, and ^{+}P represent singlet and triplet states and cation radical species of zinc porphyrin for **1**·Zn, respectively. MV represents **2**. See supporting information for details. Mataga, N.; Karen, A.; Okada, T.; Nishitani, S.; Kurata, N.; Sakata, Y.; Misumi, S. *J. Phys. Chem.* **1984**, *88*, 5138.

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