Photoinduced Electron Transfer between Zinc Protoporphyrin Reconstituted Myoglobin and Methylviologen

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Synopsis. Photoinduced electron transfer proceeded between zinc protoporphyrin reconstituted myoglobin and methylviologen. The reduced methylviologen formed by electron-transfer quenching was observed by means of laser flash photolysis. The quenching rate constants were affected by the change in the surface charges of myoglobin.

Zinc porphyrins have longer excited triplet state lifetimes compared with other metalloporphyrins such as Fe(II), Ni(II), and Cu(II) porphyrins. Therefore, the excited triplet states of zinc porphyrins are active as a photosensitizer,[1] and then are useful as a photochemical probe to study photoinduced electron transfer. Zinc porphyrin substituted for the native heme can also be used as a photochemical probe in biological systems. The photochemical properties and photoinduced energy and/or electron transfer, for example, have been studied in zinc cytochrome c,[2] zinc cytochrome c peroxidase,[3] zinc myoglobin,[4] and zinc hemoglobin.[5]

In this work, we report that photoinduced electron transfer between zinc protoporphyrin reconstituted myoglobin (ZnPP–Mb) and methylviologen takes place. The transient absorption of reduced methylviologen was observed when the sample solution containing ZnPP–Mb and methylviologen was excited with a laser pulse. This is the first direct evidence of electron transfer between the excited triplet state of ZnPP–Mb and methylviologen. The reaction rate constant was strongly affected by the change of the surface charges of ZnPP–Mb.

Experimental

Myoglobin from horse skeletal muscle was obtained from Sigma Chem. Co., and was used without further purification. Zinc protoporphyrin IX (ZnPP) was purchased from Porphyrin Products, Inc. ZnPP reconstituted myoglobin (ZnPP–Mb) was prepared according to the literature,[4f,6] except that the reconstitution of the apomyoglobin was performed in 50 mM (1 M=1 mol dm^{-3}) Tris-HCl buffer, pH 8.5. After the reconstitution, ZnPP–Mb was purified by ion-exchange DEAE-Sepharose Fast Flow chromatography in 50 mM Tris-HCl buffer, pH 8.5. ZnPP–Mb adsorbed on the column was eluted by applying a linear gradient of NaCl. ZnPP–Mb fractions possessing $A_{428}/A_{280}>9.0$ were combined and dialyzed against 10 mM phosphate or 10 mM Tris-HCl buffer at a desirable pH. The concentration of ZnPP–Mb was determined by the absorbance at 280 nm ($\varepsilon=1.58\times10^4$ M^{-1} cm^{-1})[4f]. The absorption peaks for ZnPP–Mb prepared were at 596, 554, 428, 355, and 279 nm. These values were in good agreement with those reported previously.[4c]

A Nd-YAG laser (Quanta Ray GCR-3) was used for the laser flash photolysis. The wavelength of the laser pulse was 532 nm, and the pulse duration was about 20 ns. A Xe-lamp (ILC Technology, LX-300) was used as a monitor light. The decay of the transient absorption was measured on a digital storage oscilloscope (Tektronix 11401). The signal was averaged if necessary, and was analyzed on the oscilloscope. The sample solution was deaerated by freeze-pump-thaw cycles to remove dissolved oxygen for the laser flash photolysis. The absorption spectrum of ZnPP–Mb was not changed by the freeze-pump-thaw cycles.

Results and Discussion

The triplet–triplet (T–T) absorption spectrum as shown in Fig. 1-(A) was obtained when ZnPP–Mb was excited by a 532 nm laser pulse. The decay of the transient spectrum obeyed first order kinetics. The lifetime of the transient species was 15 ms. This value was in good agreement with the reported lifetime of the excited triplet state of ZnPP–Mb (^{3}ZnPP–Mb*).[4b,c,d,5c]

When ZnPP–Mb was excited in the presence of

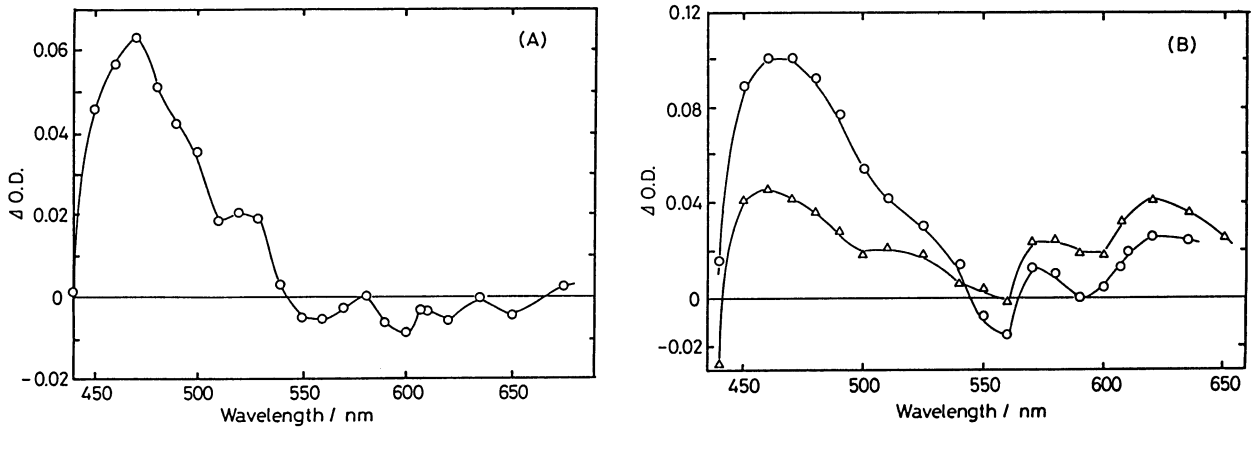

Fig. 1. Transient absorption spectrum obtained when ZnPP–Mb (2.0×10^{-5} M) was excited (A) in the absence of, and (B) in the presence of MV^{2+} (4.4×10^{-4} M) in 10 mM phosphate buffer (pH 7.0), 25 °C. The spectra were observed at (A) 50 µs, (B) (○) 30 µs, (△) 130 µs after the excitation.

MV^{2+}, the time resolved transient absorption spectra were obtained as shown in Fig. 1-(B). These were observed at 30 and 130 μs after the excitation. A new absorption band around at 610 nm was observed in addition to the T–T absorption of ZnPP-Mb. This absorption band is characteristic of reduced methylviologen (MV^+).[7] The intensity of this band increased with time accompanied with the decrease of the T–T absorption in the intensity. The lifetime of ^{3}ZnPP-Mb* decreased when MV^{2+} was added to ZnP-Mb. This means that ^{3}ZnPPMb* was quenched by MV^{2+}.

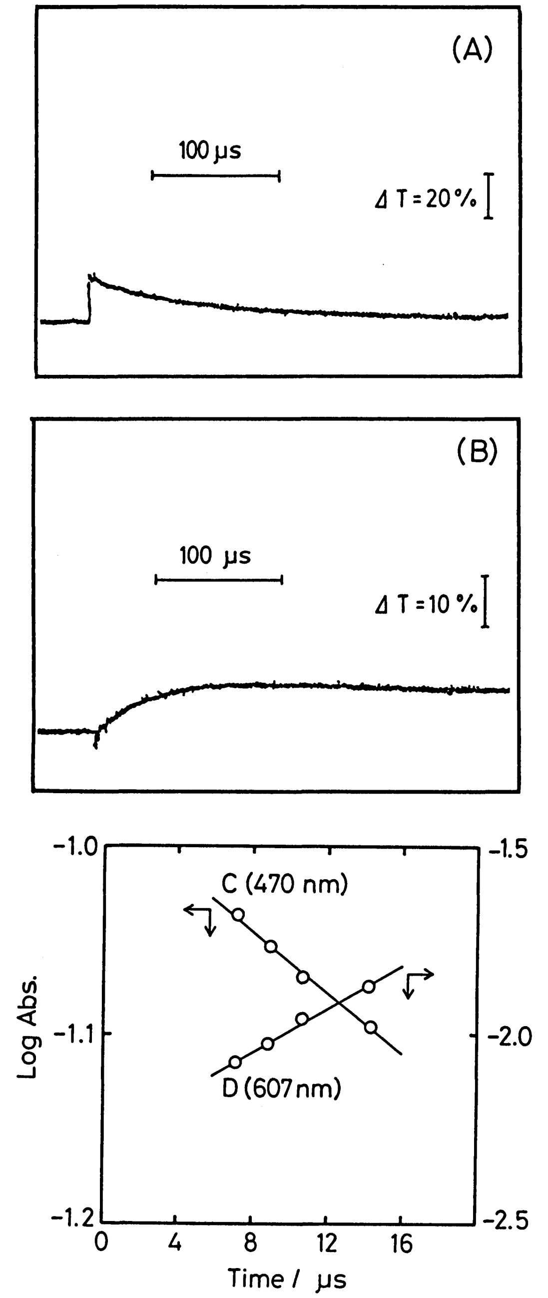

Fig. 2. (A), (B) Typical oscillograms of the transient absorption observed at (A) 470 nm and (B) 607 nm. Conditions: ZnPP-Mb (2.0×10^{-5} M), MV^{2+} (2.9×10^{-4} M) in 10 mM phosphate buffer (pH 7.0), 25 °C.; (C), (D) semi-logarithmic plots of transient absorptions.

Typical oscillograms of the transient absorption are shown in Fig. 2-(A) and (B) observed at 470 and 607 nm, respectively. ^{3}ZnPP-Mb* and MV^+ are the predominant species at 470 and 607 nm, respectively. Fig. 2 shows that the decay of ^{3}ZnPP-Mb* corresponds to the formation of MV^+, i.e., MV^{2+} quenches ^{3}ZnPP-Mb* by electron transfer to form MV^+ (Eq. 1).

$$^3\text{ZnPP-Mb}^* + \text{MV}^{2+} \xrightarrow{k_{\text{obsd}}} \text{ZnPP-Mb}^+ + \text{MV}^+ \qquad (1)$$

Although it was reported that the quenching of ^{3}ZnPP-Mb* took place by MV^{2+}, there was no direct evidence of electron-transfer quenching.[5d] The results shown in Fig. 2 are the direct evidence of electron-transfer quenching of ^{3}ZnPP-Mb* by MV^{2+}. The decay of the T–T absorption and the formation of MV^+ were also single exponential in the presence of MV^{2+} as shown in Fig. 2-(C) and (D). The observed quenching rate constant (k_{obsd} in Eq. 1) was 2.7×10^7 $M^{-1}s^{-1}$ in 10 mM phosphate buffer, pH 7.0 at 25 °C.[8]

The value of k_{obsd} was pH-dependent. The value of k_{obsd} increased with increasing pH as shown in Fig. 3. This curve is similar in shape to the titration curve of an acid-base equilibrium in which the pK_a is about 7.8. This suggests that the ionization of amino acid residues plays an important role in the pH dependence of k_{obsd}. Judging from the apparent pK_a value, lysine or histidine residues should be responsible. The acidic amino acid residues seem to be deprotonated under the conditions in Fig. 3. So, the charges on ZnPP-Mb are changed by the change in the degree of the dissociation of the basic amino acid residues with pK_a values of about 7.8.

The value of k_{obsd} was also dependent on the ionic strength as shown in Fig. 4. The value of k_{obsd} decreased with increasing ionic strength at pH 7.0 and 8.2, but increased with increasing ionic strength at pH 6.5. This may have been caused by the change of charges of ZnPP-Mb. To estimate the charges of ZnPP-Mb, the logarithm of k_{obsd} was plotted against $I^{1/2}/(1+8I^{1/2})$, where I is the ionic strength. The plots were based on the Brønsted-Debye-Hückel expression[9] (Eq. 2) in which A and B are 0.509 and 0.329×10^8

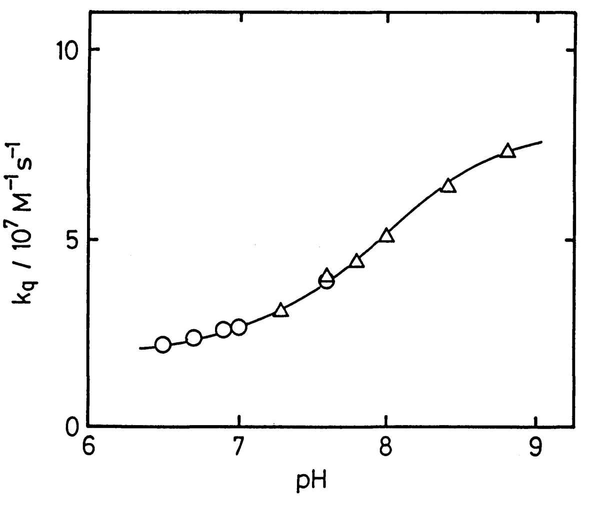

Fig. 3. The dependence of k_q on pH. Conditions. ZnPP-Mb (2.0×10^{-5} M) in (○) 10 mM phosphate buffer, (△) 10 mM Tris-HCl buffer, 25 °C.

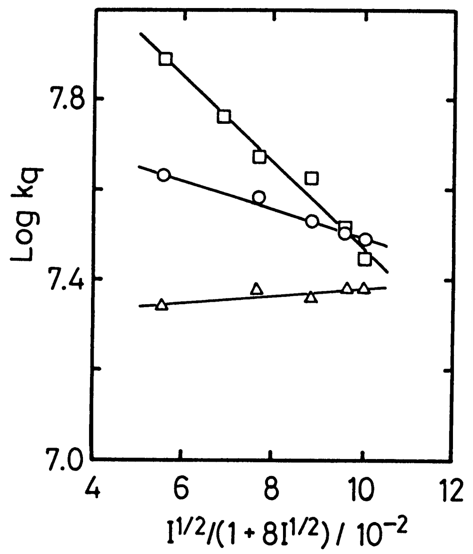

Fig. 4. Plot of log k_q as a function of $I^{1/2}/(1+8I^{1/2})$ at different pH. Conditions: ZnPP-Mb (2.0×10^{-5} M) in 10 mM phosphate buffer ((△) pH 6.5, (○) pH 7.0), and in 10 mM Tris-HCl buffer ((□) pH 8.2). The ionic strength was adjusted by NaCl.

$$\log k_{\mathrm{obsd}} = \log k_0 + 2AZ_1Z_2I^{1/2}/(1+BaI^{1/2}) \qquad (2)$$

$M^{1/2}$ cm^{-1}, respectively, in water at 25 °C; Z_1 and Z_2 are the charges of MV^{2+} and ZnPP-Mb, respectively. For Z_1 and a (the distance of closest approach between the reactants (center to center)), values of +2 and 2.50 nm[10] were used, respectively. From the slope of the lines in Fig. 4, the charges of ZnPP-Mb were estimated to be −4.7, −1.6, and +0.4 at pH 8.2, 7.0, and 6.5, respectively. The negative charges of ZnPP-Mb increased with increasing pH. ZnPP-Mb is negatively charged under the condition that the basic amino acid residues with apparent pK_a values of about 7.8 are protonated by 50% (when the pH is 7.8). These charges estimated here seem to be ones at a specific reaction site[15,16] (presumably around the entrance of the heme pocket).

The dependence of k_{obsd} on pH can be explained by the change of the charges of ZnPP-Mb. As the deprotonation of the basic amino acid residues takes place by raising the pH, the positive charges decrease, i.e., the negative charges of ZnPP-Mb increase at higher pH. The change of the charge of ZnPP-Mb makes the degree of the electrostatic interaction between ZnPP-Mb and MV^{2+} change. The more negatively charged is ZnPP-Mb, the stronger is the electrostatic interaction with MV^{2+}, because MV^{2+} is positively charged. So, the value of k_{obsd} increases with increasing pH because of the increasing of the electrostatic interaction.

References

1) a) M.-C. Richoux, "Photogeneration of Hydrogen," ed by A. Marriman and M. A. West, Academic Press, New York (1982), pp. 39—65, and references therein. b) S. Aono, I. Okura, and A. Yamada, *J. Phys. Chem.*, **89**, 1593 (1985). c) I. Okura, N. Kaji, S. Aono, T. Kita, and A. Yamada, *Inorg. Chem.*, **24**, 451 (1985).

2) a) J. M. Vanderkooi, F. Adar, and M. Erecinska, *Eur. J. Biochem.*, **64**, 381 (1976). b) B. P. Sudha, N. Dixit, V. T. Moy, and J. M. Vanderkooi, *Biochemistry*, **23**, 2103 (1984). c) H. Koloczek, T. Morie, T. Yonetani, H. Anni, G. Maniara, and J. M. Vanderkooi, *ibid.*, **26**, 3142 (1987). d) K. T. Conklin and G. McLendon, *Inorg. Chem.*, **25**, 4806 (1986). e) G. McLendon, J. R. Winkler, D. G. Nocera, M. R. Mauk, A. G. Mauk, and H. B. Gray, *J. Am. Chem. Soc.*, **107**, 739 (1985). f) H. Elias, M. H. Chou, and J. R. Winkler, *ibid.*, **110**, 429 (1988). g) L.-H. Zang and A. H. Maki, *ibid.*, **112**, 4346 (1990).

3) a) N. Liang, C. H. Kang, P. S. Ho, E. Margoliash, and B. M. Hoffman, *J. Am. Chem. Soc.*, **108**, 4665 (1986). b) P. S. Ho, C. Sutoris, N. Liang, E. Margoliash, and B. M. Hoffman, *ibid.*, **107**, 1070 (1985). c) N. Liang, G. J. Pielak, A. G. Mauk, M. Smith, and B. M. Hoffman, *Proc. Natl. Acad. Sci. U. S. A.*, **84**, 1249 (1987). d) N. Liang, A. G. Mauk, G. J. Pielak, J. A. Johnson, M. Smith, and B. M. Hoffman, *Science (Washington, D. C.)*, **240**, 311 (1988). e) S. A. Wallin, E. D. Stemp, A. M. Everest, J. M. Nocek, T. L. Netzel, and B. M. Hoffman, *J. Am. Chem. Soc.*, **113**, 1842 (1991).

4) a) J. Feitelson and T. G. Spiro, *Inorg. Chem.*, **25**, 861 (1986). b) N. Barboy and J. Feitelson, *Biochemistry*, **26**, 3240 (1987). c) S. Papp, J. M. Vanderkooi, C. S. Owen, G. R. Moltom, and C. M. Phillips, *Biophys. J.*, **58**, 177 (1990). d) N. Barboy and J. Feitelson, *Biochemistry*, **28**, 5450 (1989). e) J. A. Cowan and H. B. Gray, *Inorg. Chem.*, **28**, 2074 (1989). f) A. W. Axup, M. Albin, S. L. Mayo, R. J. Crutchley, and H. B. Gray, *J. Am. Chem. Soc.*, **110**, 435 (1988). g) R. J. Crutchley, W. R. Ellis, and H. B. Gray, *ibid.*, **107**, 5002 (1985). h) R. J. Crutchley. W. R. Ellis, and H. B. Gray, "Frontiers in Bioinorganic Chemistry," ed by A. V. Xavier, VCH: Weinheim, FRG (1986), pp. 679—693.

5) a) R. S. Moog, A. Kuki, M. D. Fayer, and S. G. Boxer, *Biochemistry*, **23**, 1564 (1984). b) J. J. Leonard, T. Yonetani, and J. B. Callis, *ibid.*, **13**, 1460 (1974). c) H. Zemel and B. M. Hoffman, *J. Am. Chem. Soc.*, **103**, 1192 (1981). d) K. P. Simolo, G. L. McLendon, M. R. Mauk, and A. G. Mauk, *ibid.*, **106**, 5012 (1984). e) J. L. McGourty, N. V. Blough, and B. M. Hoffman, *ibid.*, **105**, 4470 (1983). f) S. E. Peterson-Kennedy, J. L. McGourty, and B. M. Hoffman, *ibid.*, **106**, 5010 (1984). g) D. J. Gingrich, J. M. Nocek, M. J. Natan, and B. M. Hoffman, *ibid.*, 7533 (1987). h) M. J. Natan and B. M. Hoffman, *ibid.*, **111**, 6468 (1989). i) M. J. Natan, D. Kuila, W. W. Baxter, B. C. King, F. M. Hawkridge, and B. M. Hoffman, *ibid.*, **112**, 4081 (1990).

6) F. W. J. Teale, *Biochim. Biophys. Acta*, **35**, 543 (1959).

7) I. Okura, S. Nakamura, N. K. Thuan, and K. Nakamura, *J. Mol. Catal.*, **6**, 261 (1979).

8) The value of k_q was calculated from the Stern-Volmer plot for the lifetime of ^{3}ZnPP-Mb*. All of the plots were linear under the reaction conditions in this work.

9) A. A. Frost and R. G. Pearson, "Kinetics and Mechanisms," 2 nd ed, John Wiley & Sons, Inc., (1961), p. 150.

10) The distance of closest approach between ZnPP-Mb and MV^{2+} was assumed to be 2.5 nm according to crystallographic estimations of the sizes of myoglobin[11] and methylviologen[12] as reported for cytochrome c[13] and hemoglobin[14].

11) T. Takano, *J. Mol. Biol.*, **110**, 537 (1977).

12) J. M. Russell and S. C. Wallwork, *Acta Crystallogr., Sect. B*, **28**, 1527 (1972).

13) R. Margalit and A. Schejter, *Eur. J. Biochem.*, **32**, 492 (1973).

14) J. G. Beetlestone and D. H. Irvine, *Proc. R. Soc. London, Ser. A*, **277**, 401 (1964).

15) M. A. Cusanovich, *Bioorg. Chem.*, **IV**, 117 (1978),

16) N. Sutin, *Bioinorg. Chem.*, **II**, 156 (1977).