Photoinduced Electron Transfer from Zinc Porphyrin to a Linked Quinone in Myoglobin

Takashi Hayashi,* Tetsuo Takimura, Takafumi Ohara, Yutaka Hitomi and Hisanobu Ogoshi*

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

Myoglobin reconstituted with the covalently linked zinc porphyrin–peptide–quinone triad **1** is prepared and the singlet electron transfer from zinc porphyrin to quinone within the protein is examined by fluorescence measurements.

Over the last decade, the biological electron transfer (ET) reactions of photosynthesis and respiratory systems have attracted the interest of chemists and biochemists. Several groups have reported the mechanisms of ET process in modified proteins such as myoglobin or cytochrome c by site-directed mutagenesis to clarify the function of amino acid residue.[1] In contrast, we have designed a new model for the ET reaction in modified myoglobin by reconstitution with a zinc porphyrin bearing a quinone as an electron acceptor **1** (ZnP–Gly_n–Q, n = 0–2) (Fig. 1).[2]† In this paper, we report the preparation of the reconstituted myoglobin and the intramolecular photoinduced ET reaction between the zinc porphyrin and quinone moieties.[3]

The zinc porphyrin **1**‡ was incorporated into apomyoglobin (rMb) from horse heart.[4,5] The visible spectra of the reconstituted proteins in the region of the soret and Q bands at 415, 544 and 582 nm are quite similar to those of a reference protein reconstituted with 1,2,3,4-tetraethyl-5,8-dimethyl-6,7-di-(methoxycarbonylethyl)porphyrin zinc complex **2**. The time-of-flight mass spectrum for rMb(**1c**) shows the two significant parent peaks at 17998 and 17058, which are assigned as rMb(**1c**) and apomyoglobin, respectively, since the molecular weight of **1c** is 965.4.§

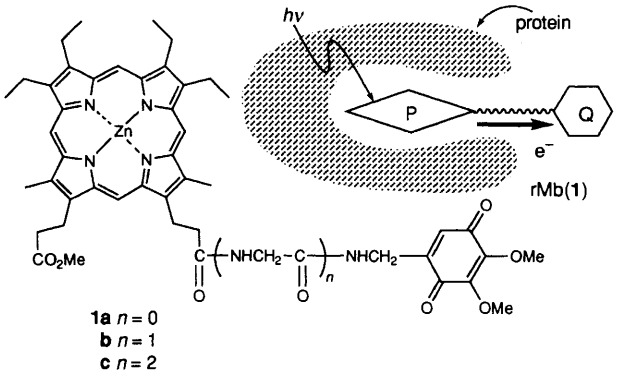

Fig. 1

The photoinduced ET reaction from zinc porphyrin to quinone in rMb(**1**) was monitored by fluorescence spectroscopy of the zinc porphyrin moiety of **1** in phosphate buffer (pH 7.0). The relative fluorescence intensities of rMb(**1**), based on the spectrum of the reference myoglobin reconstituted with zinc porphyrin **2**, decreased and a shorter fluorescence lifetime compared with reference zinc porphyrin was observed, as shown in Table 1. The fluorescence decays for rMb(**1a**), rMb(**1b**) and rMb(**1c**) exhibit double exponential curves and the lifetime of the longer component is consistent with that of the reference myoglobin without a quinone acceptor as shown in Fig. 2.¶ In contrast, the fluorescence quenching of rMb(**2**) in the presence of excess of coenzyme Q_0, 2,3-dimethoxy-5-methyl-*p*-benzoquinone, was not detected and single exponential curve of fluorescence decay with τ = 1.9 ns was obtained. These results suggest that the intramolecular singlet ET reaction from photoexcited zinc porphyrin to covalently linked quinone takes place in the system.[6] To our knowledge, no such singlet ET reaction has been reported for zinc myoglobin, although intermolecular triplet ET reactions controlled by diffusion from photoexcited zinc myoglobin to small acceptors, such as quinones, have been reported by several groups.[7] The ET rate constants estimated from the fluorescence lifetimes indicate no significant differences between **1a**, **1b** and **1c** in the protein, although the length of linker in **1b** and **1c** is longer by one glycine unit than that in **1a** and **1b**, respectively.‖ Thus, in rMb (**1b**) and rMb(**1c**), it is likely that the linker units are not completely stretched out toward the outside of protein due to the flexibility of glycine linker and the distances between donor and acceptor are almost the same in both reconstituted proteins. This study shows that the intramolecular singlet ET reactions

Table 1 Fluorescence lifetimes and rate constants of the ET reaction for reconstituted myoglobin[a]

Myoglobin	Shorter lifetime[b] τ_1/ns	Rate constant k_{ET}/s^{-1}
rMb(**1a**)	0.5[c,d]	1.5×10^9
rMb(**1b**)	0.6[c,e]	1.1×10^9
rMb(**1c**)	0.6[c,f]	1.1×10^9
rMb(**2**)	1.9[g]	
rMb(**2**) + Q_0[h]	1.9[g]	

[a] In aqueous phosphate buffer (10 mmol dm^{-3}) pH 7.0 and at 25 °C. The lifetime data were obtained on a Hamamatsu Picosecond Fluorescence Measurement System type C4780 equipped with a N_2 dye laser, LN100C2, Laser Photonics INC (λ_{ex} = 543 nm). [b] Errors in τ_1 are < 10%. [c] Data were satisfactorily analysed by the double exponential equation, $If(t) = A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2)$, using the single-photon-counting data of 560–670 nm. [d] Longer lifetime (τ_2) = 2.0 ns. $A_1:A_2$ = 45:55. [e] Longer lifetime (τ_2) = 1.9 ns. $A_1:A_2$ = 53:47. [f] Longer lifetime (τ_2) = 2.1 ns. $A_1:A_2$ = 75:25. [g] Single exponential decay was observed. [h] [Q_0]/[rMb(**2**)] = 20.

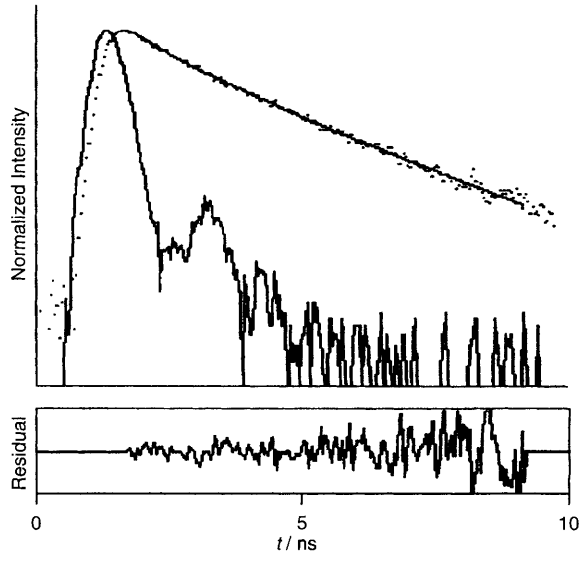

Fig. 2 Fluorescence decay profiles obtained in the range of 560–670 nm from *ca.* 10^{-6} mol dm^{-3} solutions of rMb(**1c**) at room temp. in phosphate buffer (pH 7.0), excitation wavelength = 543 nm by N_2 dye laser. The experimental data points are represented by the dots; the solid curve is the fit to two exponential functions with τ_1 = 0.6 ns and τ_2 = 2.1 ns.

between the zinc porphyrin and quinone moieties of **1** spaced by the protein proceed at slower rate constants (k_{ET} = *ca.* 10^9 s^{-1}), compared with that (k_{ET} > 10^{10} s^{-1}) of the ET reaction of **1** in CH_2Cl_2.[2]

This work was supported by a Grant-in-Aid for Specially Promoted Research (No. 04101003) from the Ministry of Education, Science and Culture, Japan. We thank Mr Akihiko Kusai of Analytical Instruments Division, JEOL Ltd. for the measurement of time-of-flight mass spectra.

Received, 8th August 1995; Com. 5/05311K

Footnotes

† Recently, we have also reported the ET reaction in **1** and the ET rate constants in CH_2Cl_2 and Me_2SO.

‡ Compounds **1a–c** and **2** were characterized by ^1H NMR and HRMS. Experimental details of the syntheses and characterizations of these compounds will be provided in forthcoming paper.

§ The paramagnetic ^1H NMR spectrum for reference myoglobin reconstituted with 1,2,3,4-tetraethyl-5,8-dimethyl-6-[2′-(methoxyglycinocarbonyl)ethyl]-7-methoxycarbonylethylporphyrin iron complex in the presence of potassium cyanide shows the characteristic proton resonances which support the normal interaction between hemin and protein. Representative proton signals of major components 21.4 (5-CH_3 of porphyrin) δ −1.9, −2.4 and −3.8 (Ile99 of protein) [phosphate buffer (D_2O), pH 7.0].[8]

¶ Part of covalently linked quinone group in the reconstituted myoglobins decomposed during the measurement, thus fluorescence decay of zinc porphyrin without any ET processes was also monitored in the protein.

‖ The rate constants of ET reaction, k_{ET}, were determined by analyses of the following fluorescence decay profiles: rMb(**1a**) τ_1 = 0.5; rMb(**1b**) τ_1 = 0.6; rMb(**1c**) τ_1 = 0.6; rMb(**2**) τ_2 = 1.9 ns at 25 °C in phosphate buffer (pH 7.0); $k_{ET} = 1/\tau_1 - 1/\tau_2$.

References

1 Recent reviews: H. B. Gray and J. R. Winkler, *Pure Appl. Chem.*, 1992, **64**, 1257; J. R. Winkler and H. B. Gray, *Chem. Rev.*, 1992, **92**, 369; B. M. Hoffman, M. J. Natan, J. M. Nocek and S. A. Wallin, in *Structure and Bonding*, Springer, Hidelberg, 1991, vol. 75, pp. 85–108; G. McLendon, in *Structure and Bonding*, Springer, Hidelberg, 1991, vol. 75, pp. 159–174.

2 T. Hayashi, T. Takimura, Y. Hitomi, T. Ohara and H. Ogoshi, *J. Chem. Soc., Chem. Commun.*, 1995, 545.

3 Photoinduced ET from covalently linked ruthenium complex to iron porphyrin in myoglobin: I. Hamachi, S. Tanaka and S. Shinkai, *J. Am. Chem. Soc.*, 1993, **115**, 10458; I. Hamachi, S. Tanaka and S. Shinkai, *Chem. Lett.*, 1993, **8**, 1417.

4 F. W. Teale, *Biochim. Biophys. Acta.*, 1959, **35**, 543; T. Yonetani and T. Asakura, *J. Biol. Chem.*, 1969, **244**, 4580.

5 Reconstituted myoglobin: H. Ogoshi, K. Kawabe, S. Mitachi, Z.-i. Yoshida, K. Imai and I. Tyuma, *Biochim. Biophys. Acta.*, 1979, **581**, 266; K. Kawabe, K. Imaizumi, K. Imai, I. Tyuma, H. Ogoshi, T. Iwahara and Z.-i. Yoshida, *J. Biochem.*, 1982, **92**, 1703; Y. Aoyama, K. Aoyagi, H. Toi and H. Ogoshi, *Inorg. Chem.*, 1983, **22**, 3046; H. Toi, M. Homma, A. Suzuki and H. Ogoshi, *J. Chem. Soc., Chem. Commun.*, 1985, 1791; A. Suzuki, K. Okuda, K. Kawagoe, H. Toi, Y. Aoyama and H. Ogoshi, *Chem. Lett.*, 1985, 1169.

6 A. Shiemiarczuk, A. R. McIntosh, T.-F. Ho, M. J. Stillman, K. J. Roach, A. C. Weedon, J. R. Bolton and J. S. Connolly, *J. Am. Chem. Soc.*, 1983, **105**, 7224; J. A. Schmidt, A. R. McIntosh, A. C. Weedon, J. R. Bolton, J. S. Connolly, J. K. Hurley and M. R. Wasielewski, *J. Am. Chem. Soc.*, 1988, **110**, 1733; D. Gust, T. A. Moore and A. L. Moore, *Acc. Chem. Res.*, 1993, **26**, 198.

7 N. Barboy and J. Feitelson, *Biochemistry*, 1987, **26**, 3240; N. Barboy and J. Feitelson, *Biochemistry*, 1989, **28**, 5450; J. Feitelson and G. McLendon, *Biochemistry*, 1991, **30**, 5051; S. Aono, S. Nemoto and I. Okura, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 591; K. Tsukahara and M. Okada, *Chem. Lett.*, 1992, 1543; K. Tsukahara, S. Asami, M. Okada and T. Sakurai, *Bull. Chem. Soc. Jpn.*, 1994, **67**, 471; K. Tsukahara, M. Okada, S. Asami, Y. Nishikawa, N. Sawai and T. Sakurai, *Coord. Chem. Rev.*, 1994, **132**, 223.

8 G. N. La Mar, N. L. Davis, D. W. Parish and K. M. Smith, *J. Mol. Biol.*, 1983, **168**, 887.