CHEMISTRY LETTERS, pp. 1543-1546, 1992.

Electron-Transfer Quenching and Thermal Backward Electron-Transfer Reactions of Zinc Myoglobin Controlled by Conformational Change

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Both photoinduced electron-transfer and thermal backward electron-transfer reactions of zinc myoglobin with cationic quenchers were bimolecular steps and the reaction rates were insensitive to the driving force of reactions, suggesting that the reactions are controlled by conformational changes of zinc myoglobin.

Electron-transfer (ET) reaction theories such as the Marcus theory have been applied to many ET reactions concerning metalloproteins.[1)] A number of reaction systems have shown the driving force dependence of the ET rate constant. Recently the other factor controlling the ET rate, such as conformational changes (a gating mechanism),[2)] has been proposed in the ET reactions of cytochrome c[3)] and zinc-substituted cytochrome c,[4)] hemoglobin,[5)] and myoglobin.[5-7)] In the case of zinc myoglobin, however, direct evidence for the ET reaction, such as the detection of thermal backward ET, has not been observed.[8)] In this letter, we provide direct evidence for the ET quenching controlled by a conformational change by using a variety of cationic quenchers (Q^{n+}).

Horse heart muscle metmyoglobin (Sigma) was reconstituted with Zn(II)protoporphyrinIX in a previously reported method.[10,11)] A single flash photolysis was carried out in a degassed solution containing 3.0×10^{-6} M zinc myoglobin (ZnPPMb; 1 M = 1 mol dm^{-3}) and cationic quenchers (0-2.0×10^{-5} M), such as a hexaammineruthenium(III) ion ($[Ru(NH_3)_6]^{3+}$), viologen (ETQ^{4+}, PTQ^{4+}, BTQ^{4+}, and CMV^{+}),[12)] and a Cu(II) protein (stellacyanin), at 25 °C, pH 7.0, and the various ionic strengths (I) by using a Photal RA-412 pulse flash apparatus with a 30 μs pulse-width Xe lamp ($\lambda > 450$ nm). Absorption spectral changes during the reaction were monitored at 428, 460, and 680 nm. One of the examples is shown in Fig. 1. The decay of the excited triplet state of ZnPPMb (*ZnPPMb) monitored at 460 nm was monophasic and the rate was first-order in the concentrations of

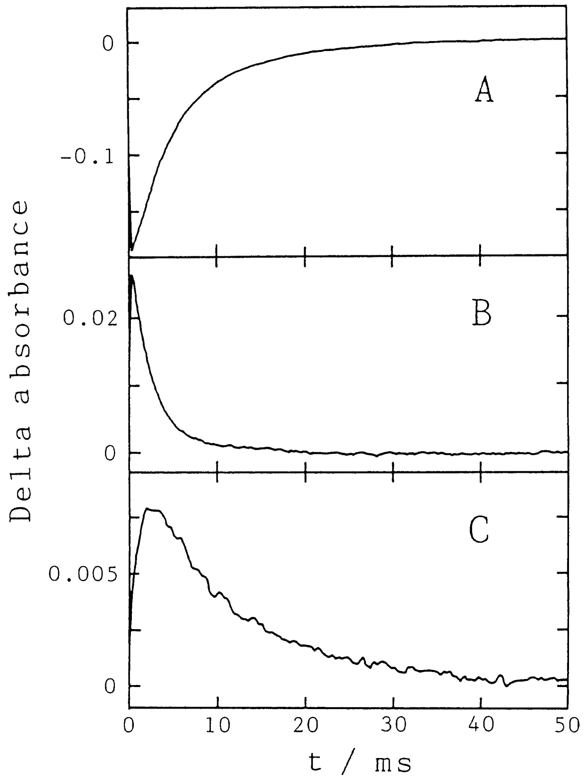

Fig. 1. Absorbance changes following production of *ZnPPMb by irradiation of ZnPPMb with a Xe-flash lamp in the presence of CMV^{+} ion (1.20×10^{-5} M) at pH 7.0 (a 10 mM phosphate buffer) and I = 0.02 M. (A) 428 nm, (B) 460 nm, and (C) 680 nm.

both *ZnPPMb and the quencher. The absorption spectral changes at 428 and 680 nm were biphasic (fast and slow) for all of the cationic quencher systems examined in this work. The slow phase monitored at 680 nm was consistent with second-order kinetics. The absorption at 680 nm might be mainly due to a porphyrin radical cation ($ZnPPMb^{+}$). Therefore, the biphasic behavior can be attributed to the formation of the radical cations ($ZnPPMb^{+}$ and $Q^{(n-1)+}$) and the thermal backward ET reaction. The reaction scheme can be represented as

$$\mathrm{ZnPPMb} \underset{k_0}{\overset{h\nu}{\rightleftarrows}} {}^{*}\mathrm{ZnPPMb} \qquad (1)$$

$$^{*}\mathrm{ZnPPMb} + Q^{n+} \xrightarrow{k_q} \mathrm{ZnPPMb}^{+} + Q^{(n-1)+} \qquad (2)$$

$$\mathrm{ZnPPMb}^{+} + Q^{(n-1)+} \xrightarrow{k_b} \mathrm{ZnPPMb} + Q^{n+} \qquad (3)$$

The rate constants for both photoinduced ET and thermal backward ET reactions (k_q and k_b) are listed in Table 1.

In the reactions of bis(viologens), ETQ^{4+}, PTQ^{4+}, and BTQ^{4+}, a dis-

Table 1. The Rate Constants for the Photoinduced ET and Thermal Backward ET Reactions (k_q and k_b) at 25 °C and I = 0.02 M

Quencher	E^0/V	k_q/M^{-1} s^{-1}	k_b/M^{-1} s^{-1}
$[Fe(CN)_6]^{3-}$ a)	0.36	intracomplex ET	
stellacyanin b)	0.18	3.5×10^6	5.9×10^6
$[Ru(NH_3)_6]^{3+}$	0.05	5.0×10^7	2.6×10^7
O_2 c)	-0.16	1.3×10^8	—
ETQ^{4+}	-0.28	1.3×10^7	3.3×10^7
PTQ^{4+}	-0.33	3.2×10^6	2.4×10^7
AQS^- d,e)	-0.38	2.9×10^8	—
BTQ^{4+}	-0.39	7.5×10^6	2.4×10^7
CMV^+	-0.42	2.6×10^7	1.1×10^8
MV^{2+}	-0.44	4.5×10^7 e)	4.0×10^7

a) Ref. 11. b) Ref. 13. c) Ref. 10. d) Anthraquinone-2-sulfonate. e) Ref. 6.

proportionation reaction of the monoradical cation ($Q\text{-}Q^{3+}$) is much faster than the backward ET reaction.14) Therefore, the backward ET reaction must correspond to that of the diradical cation ($Q\text{-}Q^{2+}$):

$$2Q\text{-}Q^{3+} \longrightarrow Q\text{-}Q^{2+} + Q\text{-}Q^{4+} \qquad \text{fast} \qquad (4)$$

$$ZnPPMb^+ + Q\text{-}Q^{2+} \xrightarrow{k_b} ZnPPMb + Q\text{-}Q^{3+} \qquad (5)$$

Both k_q and k_b values are in a narrow range for a variety of quenchers and are smaller than the diffusion-controlled limit. Application of the Marcus theory was unsuccessful for these systems. A larger value of k_q was obtained for a small and neutral molecule (O_2) or an anionic quencher (AQS^-). A hexacyanoferrate(III) ion ($[Fe(CN)_6]^{3-}$), which is a highly charged anion, forms a self-associated complex with ZnPPMb and an efficient intracomplex photoinduced ET reaction occurs.11) The k_q values tend to decrease with increasing the charge of cationic quenchers. The positively charged and large Cu(II) protein, stellacyanin, gave a small k_q value. From the ionic strength dependence of the k_q values for the cationic quenchers, the effective charge of *ZnPPMb is estimated to be +0.5. The active site of *ZnPPMb can be assigned to positively charged Lys or Arg residues on the surface of the protein, such as those near the heme pocket.

Barboy and Feitelson have proposed a conformational gating mechanism for the bimolecular quenching reaction of *ZnPPMb.$^{6,7)}$ Insensitivity of the k_q and k_b values to the driving force of reactions, which is found in this work, supports the gating mechanism that the rate of reaction is controlled by a conformational change of ZnPPMb associated with charge and largeness of the quenchers for not only photoinduced ET but also thermal backward ET reactions.

This research was partly supported by a Grant-in-Aid for Scientific Research (No. 03453049) from the Ministry of Education, Science and Culture, Japan and by a grant from Itoh Science Foundation.

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(Received May 7, 1992)