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Photoinduced electron–transfer reactions of zinc and magnesium myoglobins

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ABSTRACT

Photoinduced electron–transfer (ET) reaction between zinc– or magnesium myoglobin and a variety of quenchers, such as $[Ru(NH_3)_6]^{3+}$, cationic viologens, copper(II) protein (stellacyanin) and anthraquinone–2–sulfonate has been studied in an aqueous degassed solution. Not only photoinduced ET but also thermal backward ET reactions were insensitive to the driving force of reactions, suggesting that the reactions are controlled by conformational changes in myoglobins.

A. INTRODUCTION

Electron–transfer (ET) reaction theories such as the Marcus theory have been applied to many ET reactions involving metalloproteins [1]. A number of reaction systems have shown the driving force dependence of the ET rate constant. However, another factor controlling the ET rate, conformational changes of apoprotein (a gating mechanism) [2], has been recently proposed in the ET reactions of zinc–cytochrome *c* [3], hemoglobin [4] and myoglobin (ZnPPMb) [4,5]. In the present study, we shall check the gating mechanism for both ET quenching and the thermal backward ET processes for ZnPPMb and MgDPMb using a variety of cationic quenchers, such as $[Ru(NH_3)_6]^{3+}$, viologens (MV^{2+}, 1,1'–dimethyl–4,4'–bipyridinium ion; CMV^{+}, 1–methyl–1'–carboxymethyl–4,4'–bipyridinium ion; DQ^{2+}, 1,1'–ethylene–2,2'–bipyridinium ion; $MPDQ^{2+}$, 1,1'–trimethylene–4,4'–dimethyl–2,2'–bipyridinium ion; ETQ^{4+}, 1,1"–ethylenebis(1'–methyl–4,4'–bipyridinium ion; PTQ^{4+}, 1,1"–trimethylenebis(1'–methyl–4,4'–bipyridinium ion; BTQ^{4+}, 1,1"–tetramethylenebis(1'–methyl–4,4'–bipyridinium ion) and a blue copper(II) protein (stellacyanin).

B. EXPERIMENTAL

Horse heart muscle metmyoglobin (Sigma) was reconstituted with Zn(II)protoporphyrinIX (ZnPP) or Mg(II)deuteroporphyrinIX (MgDP) at 4 °C in the dark. Viologens were prepared by a previously reported method [6,7]. The sample solution was gently purged with Ar gas and then carefully degassed by freeze–pump–thaw cycles. A single flash photolysis was carried out in the deaerated solutions containing ZnPPMb or MgDPMb (0.3–5.0 μM) and quenchers (0–20 μM) at 25 °C and pH 7.0 (a 10

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mM phosphate buffer) with various ionic strengths (I) using a Photal RA-412 pulse flash apparatus with a 30 μs pulse-width Xe lamp (λ > 450 nm). Absorption spectral changes during the reaction were monitored at 428, 460 and 680 nm for ZnPPMb and 410, 438 and 680 nm for MgDPMb. A lifetime of the fluorescence of ZnPPMb and MgDPMb were measured using a Horiba NAES-500 single photon counting system. The ionic strength of the solution was adjusted with NaCl.

C. RESULTS AND DISCUSSION

(i) Intermolecular ET quenching

The lifetimes of the excited singlet state of ZnPPMb and MgDPMb were 2.3 ns and 11.4 ns, respectively. The fluorescence was not quenched by all of the quenchers used in this work. On the other hand, the excited triplet state, whose lifetime was 13–15 ms for both myoglobins, was efficiently quenched. The absorption spectral changes at 428 and 680 nm for ZnPPMb and at 410 and 680 nm for MgDPMb were biphasic (fast and slow reactions). The decay of ^{3}ZnPPMb at 460 nm or ^{3}MgDPMb at 438 nm was monophasic and was first order for at least 85% of the decay. The values of the first-order rate constants were linearly dependent on the concentraions of the quenchers. The slow phase reaction monitored at 680 nm was consistent with second-order kinetics and the rate was independent of the concentrations of the quenchers. The absorption at 680 nm might be mainly due to a porphyrin radical cation (ZnPPMb$^+$). Therefore, the biphasic behaviour, fast and slow reactions, can be attributed to the formation of the radical cations (ZnPPMb$^+$ and $Q^{(n-1)+}$) and the thermal backward ET reaction, respectively. The photoinduced ET reactions of ^{3}ZnPPMb and ^{3}MgDPMb with the quenchers can be represented by eqns. (1)–(3) and the decays of ^{3}ZnPPMb and ZnPPMb$^+$ obey the rate laws, $-d[^3\mathrm{ZnPPMb}]/dt = (k_O + k_q[Q^{n+}])[^3\mathrm{ZnPPMb}]$ and $-d[\mathrm{ZnPPMb}^+]/dt = k_b[\mathrm{ZnPPMb}^+][Q^{(n-1)+}]$, respectively.

$$^3\mathrm{ZnPPMb} \xrightarrow{k_O} \mathrm{ZnPPMb} \tag{1}$$

$$^3\mathrm{ZnPPMb} + Q^{n+} \xrightarrow{k_q} \mathrm{ZnPPMb}^+ + Q^{(n-1)+} \tag{2}$$

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

The rate constants for both photoinduced ET and thermal backward ET reactions (k_q and k_b) are listed in Tables 1 and 2.

All of the quenchers examined in this work did not form a self-associated complex with ^{3}ZnPPMb or ^{3}MgDPMb, and the intermolecular quenching occurred. On the other hand, the intracomplex ET quenching occurs in the reaction of ^{3}ZnPPMb with a hexacyanoferrate(III) ion ($[\mathrm{Fe(CN)_6}]^{3-}$) and much faster thermal backward ET occurs in a self-associated complex [11].

(ii) Ionic strength effect

The k_q values for the quenching of ^{3}ZnPPMb or ^{3}MgDPMb by the cationic quenchers increase with increasing ionic strengths. The k_b values also increase with increasing ionic strengths. Both k_q and k_b values for the anionic quencher system (AQS$^-$) decrease with increasing ionic strengths. The ionic strength dependence on the quenching rate suggests that the reactive site of ^{3}ZnPPMb and ^{3}MgDPMb is a positively charged residue. Plots of log k_q vs the squre root of ionic strength were linear. The estimated charge of ^{3}ZnPPMb was +0.45 for $[\mathrm{Ru(NH_3)_6}]^{3+}$, +0.38 for ETQ^{4+} and

+0.63 for PTQ^{4+} systems, respectively. The similar charge of ^{3}MgDPMb was estimated: +0.35 for MV^{2+}, +0.35 for CMV^+ and +0.42 for AQS^- systems, respectively. The estimated charge of +0.5 for ^{3}ZnPPMb and of +0.4 for ^{3}MgDPMb suggest that the reactive site of both myoglobins is the same as that expected for the quenching by $[Fe(CN)_6]^{3-}$: Lys and/or Arg residue(s) near the heme pocket, the former being predominant on the surface of the protein [11].

TABLE 1
Rate constants for the quenching reactions of ^{3}ZnPPMb and ^{3}MgDPMb at 25 °C, pH = 7.0 and I = 0.02 M (a 10 mM phosphate buffer)

Quencher	E^0 [a] / V	k_q (ZnPPMb) [a] / M^{-1} s^{-1}	k_q (MgDPMb) / M^{-1} s^{-1}
stellacyanin	0.18	3.5×10^6	---
$[Ru(NH_3)_6]^{3+}$	0.05	5.0×10^7	---
O_2	-0.16	1.0×10^8 [b] 1.3×10^8 [c]	---
ETQ^{4+}	-0.28	1.3×10^7	---
PTQ^{4+}	-0.33	3.2×10^6	---
DQ^{2+}	-0.35	---	6.0×10^7
AQS^-	-0.38	2.9×10^8 [b]	2.6×10^8
BTQ^{4+}	-0.39	7.5×10^6	---
CMV^+	-0.42	2.6×10^7	3.2×10^7
MV^{2+}	-0.45	4.7×10^7 4.5×10^7 [b]	2.3×10^7
$MPDQ^{2+}$	-0.69	---	3.2×10^7

[a] Ref. 8. [b] I = 0.05 M. Ref. 9. [c] I = 0.20 M. Ref. 10.

TABLE 2
Rate constants for the thermal backward ET reactions of $ZnPPMb^+$ and $MgDPMb^+$ at 25 °C, pH = 7.0 and I = 0.02 M (a 10 mM phosphate buffer)

Reductant	E^0 [a] / V	k_b (ZnPPMb) [a] / M^{-1} s^{-1}	k_b (MgDPMb) / M^{-1} s^{-1}
stellacyanin	0.18	5.9×10^6	---
$[Ru(NH_3)_6]^{2+}$	0.05	2.6×10^7	---
PTQ^{2+}	-0.19	2.4×10^7	---
ETQ^{2+}	-0.28	3.3×10^7	---
BTQ^{2+}	-0.29	2.4×10^7	---
DQ^+	-0.35	---	4.9×10^7
AQS^{2-}	-0.38	---	1.3×10^8
CMV	-0.42	1.1×10^8	9.6×10^7
MV^+			
$MPDQ^+$	-0.69	---	3.6×10^8

[a] Ref. 8.

(iii) Effect of highly charged redox inactive ions

Although a highly charged cation, poly-L-Lys, drastically inhibited the quenching of ^{3}ZnPPMb by $[Fe(CN)_6]^{3-}$ due to a complex formation between ^{3}ZnPPMb and poly-L-Lys at low ionic strengths [11], poly-L-Lys had only a small effect on the rate of quenching by $[Ru(NH_3)_6]^{3+}$, which is due to the ionic strength effect. This is understandable on the basis of the fact that there is no evidence for complex formation between $[Ru(NH_3)_6]^{3+}$ and poly-L-Lys from absorption spectral and cyclic voltammetry measurements. The rate of quenching of ^{3}ZnPPMb by PTQ^{4+} in the presence of sodium hexametaphosphate, which is a cyclic hexamer of phosphate possessing a −6 charge, became faster than that in the absence of hexametaphosphate at low ionic strengths. At high ionic strengths there was no difference in the rate between the two. Absorption spectral measurements showed the complex formation between PTQ^{4+} and hexametaphosphate. Therefore, the acceleration of the quenching rate by hexametaphosphate is attributable to the decrease in charge repulsion between ^{3}ZnPPMb and PTQ^{4+} by the complex formation of PTQ^{4+} with hexametaphosphate.

(iv) Protein-protein reaction

Stellacyanin is a blue copper glycoprotein possessing 107 amino acids and the net charge of the protein is positive at a neutral pH (p*I* = 9.9) [12]. The rate of quenching of ^{3}ZnPPMb by stellacyanin increases with increasing ionic strength; the estimated charge of the reactive site of stellacyanin is +2.6 from the ionic strength dependence, assuming that the charge of ^{3}ZnPPMb is +0.5. Wherland et al. have discussed the reactive site of stellacyanin for the reduction by Cr(II) which is most probably bound to Asp49 [13]. They suggested that Tyr85, being adjacent to Asp49 and Lys50, is involved in the redox reactions of stellacyanin along with the partially exposed His92. The former residue is near a positive patch (Lys 54, 83 and 98); therefore, the quenching of ^{3}ZnPPMb by stellacyanin may occur between the heme pocket of ^{3}ZnPPMb and the same region of stellacyanin as that for the Cr(II) reduction.

Metmyoglobin and its imidazole adduct, the latter being much more reactive than the former upon reduction [14,15], had no effect on the spontaneous decay of ^{3}ZnPPMb.

(v) Mechanism of reaction

Both k_q and k_b values in Tables 1 and 2 are in a narrow range for a variety of quenchers and are smaller than the diffusion-controlled limit in aqueous solutions. Moreover, the rate constants for ZnPPMb are very similar to those for MgDPMb; there was no effect of the substitution of metal ions. The Marcus theory has been well applied to metmyoglobin/deoxymyoglobin systems with a variety of redox reagents [15]. Application of the Marcus theory is, however, unsuccessful for ZnPPMb and MgDPMb. The estimated self-exchange rate constants for the $ZnPPMb^{+/*}$ system based on the Marcus theory are spread out over the range from 10^{-6} to 10^3 M^{-1} s^{-1} (see Table 3). The disagreement in the values of estimated self-exchange rate constants for the $ZnPPMb^{+/0}$ system is more serious. Although the electrostatic interactions are important at low ionic strengths, we ignored the work terms in the calculation. The discrepancy in the self-exchange rate constants for the present systems cannot be explained by only the work terms. The large value of k_{ex} for the $ZnPPMb^{+/*}$ system is obtained for a small molecule (O_2) or an anionic quencher (AQS^-). The highly charged anion, $[Fe(CN)_6]^{3-}$, forms a self-associated complex with ZnPPMb and an efficient intracomplex photoinduced ET reaction occurs [11]. The values of k_{ex} for the $ZnPPMb^{+/*}$ system tend to decrease with increasing the charge of cationic quenchers. The positively charged and large Cu(II) protein, stellacyanin, gave the smallest k_{ex} value.

A variety of the estimated k_{ex} values suggests that the quenching of ^{3}ZnPPMb or

TABLE 3
Estimated self-exchange rate constants for $ZnPPMb^{+/*}$ and $ZnPPMb^{+/0}$ systems based on the Marcus theory [a]

Quencher	$k_{ex}(ZnPPMb^{+/*})$ / M^{-1} s^{-1}	Reductant	$k_{ex}(ZnPPMb^{+/0})$ / M^{-1} s^{-1}
stellacyanin	3.4×10^{-6}	stellacyanin	1.8×10^{-3}
$[Ru(NH_3)_6]^{3+}$	1.8	$[Ru(NH_3)_6]^{2+}$	4.1×10^{-2}
O_2	5.6×10^{4}	PTQ^{2+}	4.0×10^{-7}
ETQ^{4+}	4.9×10^{-2}	ETQ^{2+}	3.5×10^{-10}
PTQ^{4+}	1.1×10^{-2}	BTQ^{2+}	2.4×10^{-9}
AQS^{-}	9.0×10^{2}	CMV	2.2×10^{-10}
BTQ^{4+}	4.6×10^{-1}	MV^{+}	8.6×10^{-12}
CMV^{+}	1.6×10		
MV^{2+}	9.2×10		

[a] Calculated rate constants of the self-exchange reaction of zinc myoglobin based on the Marcus equation, $k_{12} = (k_{11}k_{22}f_{12}K_{12})^{1/2}$ and $\ln f_{12} = (\ln K_{12})^2/4\ln(k_{11}k_{22}/10^{22})$. K. Tsukahara, S. Asami, M. Okada and T. Sakaurai, unpublished results.

^{3}MgDPMb is not controlled by the ET step or that the ET quenching is nonadiabatic. The latter case is probably unlike for these systems since (i) the ET quenching and thermal backward ET reactions efficiently occur in the self-associated complex of ZnPPMb with $[Fe(CN)_6]^{3-}$ [11] and in covalently attached $[Ru(NH_3)_5]$–^{3}ZnPPMb systems [16] and (ii) the thermal ET reactions of metmyoglobins with a variety of reductants are adiabatic [15]. Barboy and Feitelson have proposed a conformational gating mechanism for the bimolecular quenching reaction of ^{3}ZnPPMb [5,9]. They have suggested a charge effect along the migratory pathway toward the ZnPP in the protein or a solvation effect for the cationic and anionic quenchers. Our results strongly suggest that both quenching and thermal backward ET reactions are controlled by a conformational change in ^{3}ZnPPMb or $ZnPPMb^+$ as well as ^{3}MgDPMb or $MgDPMb^+$. We propose the following mechanism where both ^{3}ZnPPMb and $ZnPPMb^+$ have two conformers, *active* and *inactive* forms:

$$(^3ZnPPMb)_A \xrightarrow{k_0} ZnPPMb \qquad (4)$$

$$\underset{\textit{'inactive form'}}{(^3ZnPPMb)_A} + Q^{n+} \rightleftharpoons \underset{\textit{'active form'}}{(^3ZnPPMb)_B} + Q^{n+} \qquad \text{conformational change} \qquad (5)$$

$$(^3ZnPPMb)_B + Q^{n+} \xrightarrow[\text{fast}]{\text{ET}} (ZnPPMb^+)_B + Q^{(n-1)+} \qquad (6)$$

$$(ZnPPMb^+)_B + Q^{(n-1)+} \rightleftharpoons (ZnPPMb^+)_A + Q^{(n-1)+} \qquad \text{conformational change} \qquad (7)$$

$$(ZnPPMb^+)_A + Q^{(n-1)+} \xrightarrow{k_b} ZnPPMb + Q^{n+} \qquad (8)$$

After excitation, ^{3}ZnPPMb which is referred to 'form A' converts to another active 'form B' (eqn. (5)). The quenching rate is dependent on the concentrations of Q^{n+}. Therefore, the formation of the active form B is induced by attacking of Q^{n+}. Then the ET quenching occurs in eqn. (6). If a shorter distance is required for the thermal backward ET process between $ZnPP^+$ and $Q^{(n-1)+}$ than for the quenching reaction since k_b is smaller than k_q in each system, further rearrangement in $ZnPPMb^+$ might occur to keep $ZnPP^+$ and $Q^{(n-1)+}$ at a proper distance from each other. Metmyoglobin had no effect on the spontaneous decay of ^{3}ZnPPMb. Therefore, metmyoglobin might not induce any conformational changes in ^{3}ZnPPMb.

D. CONCLUSIONS

The excited triplet state of ZnPPMb was quenched by $[Fe(CN)_6]^{3-}$ via a self-associated complex, whose rate could be controlled by adding cationic poly-L-Lys or increasing ionic strengths. The thermal backward ET reaction for this system was not observed; it is most likely that the backward ET step is much faster than the quenching reaction. All of the cationic quenchers examined in this work did not form the self-associated complex with ^{3}ZnPPMb or ^{3}MgDPMb, and the intermolecular quenching occurred. The thermal backward ET reaction was observed for these cationic quenchers. Not only photoinduced ET but also thermal backward ET reactions were insensitive to the driving force of reactions, suggesting that the reactions are controlled by conformational change in ZnPPMb or MgDPMb.

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