February, 1994] © 1994 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 67, 421—431(1994) 421

Kinetics and Mechanisms of Photoinduced Electron-Transfer Reaction of Zinc Myoglobin.
Intracomplex vs. Intermolecular Quenching Controlled by Conformational Change Associated with Charge and Steric Bulk of Quenchers

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(Received August 13, 1993)

Photoinduced electron transfer (ET) between zinc myoglobin (ZnPPMb) and a variety of quenchers, such as hexacyanoferrate(III) ($[Fe(CN)_6]^{3-}$) and hexaammineruthenium(III) ($[Ru(NH_3)_6]^{3+}$) ions, cationic viologens, copper(II) protein (stellacyanin), and metmyoglobins, has been studied in aqueous degassed solutions. The excited triplet state of ZnPPMb (*ZnPPMb) was quenched by $[Fe(CN)_6]^{3-}$ in a self-associated complex. Both quenching rate constant and formation constant of the self-associated complex decrease with 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 a self-associated complex with *ZnPPMb, 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 the reactions, suggesting that the reactions are controlled by conformational changes in ZnPPMb. The quenching rate constants increase with increasing ionic strength for the cationic quenchers. The effects of poly-L-lysine hydrochloride, sodium poly-L-glutamate, and sodium *cyclo*-hexaphosphate were also examined. The active site of the *ZnPPMb toward both anionic and cationic quenchers is assumed to be the positively charged site near the heme pocket.

Intramolecular electron-transfer(ET) reactions have received considerable attention in the fields of both chemistry and biochemistry. In some biological ET reactions including hemoproteins, the intramolecular ET occurs in a complex of two proteins which forms by an electrostatic attractive force.[1)] The electrostatic interaction is, therefore, one of the important factors controlling ET reactions of metalloproteins. Reconstituted myglobin with protoporphyrinIXato(2-)-zinc(II) (ZnPPMb) has been well characterized, and the photoinduced ET reactions with molecular oxygen, anthraquinone-2-sulfonate (AQS^-), Cu^{2+}, and methylviologen (1,1′-dimethyl-4,4′-bipyridinium chloride, $MVCl_2$) have been investigated.[2—6)] In these systems, intermolecular ET reactions have been observed. In this study, we have found that a hexacyanoferrate(III) ion ($[Fe(CN)_6]^{3-}$) forms a complex with ZnPPMb and that the photoinduced ET quenching occurs in the self-associated complex of ZnPPMb–$[Fe(CN)_6]^{3-}$.

ET reaction theories such as the Marcus theory have been applied to many ET reactions involving metalloproteins.[1a)] 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 (a gating mechanism),[7)] has been recently proposed in the ET reactions of zinc-cytochrome *c* (ZnCyt*c*),[8)] hemoglobin (ZnHb),[9)] and myoglobin (ZnPPMb).[4,9)] In this study, we shall check the gating mechanism for both ET quenching and the thermal backward ET processes using a variety of cationic quenchers, such as a hexaammineruthenium(III) ion ($[Ru(NH_3)_6]^{3+}$), viologens, metmyoglobins (metMb(X); X=H_2O and imidazole), and a blue copper(II) protein (stellacyanin). Preliminary communications have been published elsewhere.[10)]

Experimental

Reagents. Metmyoglobins from sperm whale skeletal muscle (S. W.) and horse heart muscle (H. H.) were obtained from Sigma and were purified as previously described.[11)] Recombination of protoporphyrinIXato(2-)-zinc(II) (ZnPP, Sigma) with apomyoglobin[12)] was done at 4 °C using a slightly modified method that has already been published.[2—5)] ZnPP (1.1 equiv amount of apomyoglobin) was dissolved in 0.1 M NH_3 aq and was diluted four-fold with water. The ZnPP solution was added dropwise into an aqueous apomyoglobin solution (pH 8.6), and the pH of the mixture was readjusted to 8.6 with a 0.5 M HCl solution. The solution was then left in a refrigerator at 4 °C overnight. The pH of the ZnPPMb solution was adjusted to 7.0 for S. W. ZnPPMb and 6.5 for H. H. ZnPPMb. The ZnPPMb solutions were chromatographed on a CM-52 cellulose column, and were eluted with phosphate buffer solutions (0.01—0.04 M, 1 M=1 $mol\,dm^{-3}$) at pH 7.0 and pH 6.5, respectively. This procedure was done in the dark or, when necessary, under very dim red-lamp illumination. The concentrations of ZnPPMb were measured spectrophotometrically ($\varepsilon_{428}=1.53\times10^5\ M^{-1}\,cm^{-1}$).[5)] The ZnPPMb solution, the absorption ratio of A_{428}/A_{280} of which is greater than 9.5, was used for kinetic measurements. *Rhus vernicifera* stellacyanin was isolated and purified from Chinese lacquer latex (Takano and Co., Kanazawa, Japan) according to the literature.[13,14)] The concentrations of stellacyanin were measured spectrophotometrically ($\varepsilon_{604}=4.08\times10^3\ M^{-1}\,cm^{1}$).[15)] $[Ru(NH_3)_6]Cl_3$ (Aldrich) was purified as described in the literature.[16)] Methylviologen (Tokyo Kasei) was converted to a perchlorate salt as monohydrate and recrystallized twice from water. The *N*,*N*″-polymethylenebis(1′-methyl-4,4′-bipyridinium) perchlorate, (ETQ$(ClO_4)_4$ for ethylene; PTQ$(ClO_4)_4$ for trimethylene; BTQ$(ClO_4)_4$ for tetremethylene), was prepared by a previously reported method.[17,18] Sodium poly-L-glutamate (poly-L-Glu; mean F. W. 13000, Sigma), poly-L-lysine hydrochloride (poly-L-Lys; mean F. W. 3000, Wako), and sodium *cyclo*-hexapohsphate (HMP) were used without further purification. All other chemicals used were of guaranteed grade from Wako Pure Chemical Industries, Ltd. All of the solutions were prepared from redistilled water. The ionic strength (I) of the solution was adjusted with NaCl.

Kinetic Measurements. The sample solution was gently purged with Ar gas (99.9999%) and then carefully degassed by freeze-pump-thaw cycles. The ratio of A_{428}/A_{280} was checked for each solution. A single flash photolysis was done in the deaerated solutions containing ZnPPMb ($(0.3—5.0)\times10^{-6}$ M) and quenchers ($0—7.0\times10^{-5}$ M) at 25 °C and pH 7.0 with various ionic strengths using a Photal RA-412 pulse flash apparatus with a 30 μs pulse-width Xe lamp ($\lambda>450$ nm; a Toshiba Y-47 glass filter). Absorption spectral changes during the reaction were monitored at 428, 460, and 680 nm. Whether with or without the glass filter the same kinetic behavior was observed; however, the amplitude of the absorbance with the filter was 1/3 of that without it.

Other Measurements. Cyclic voltammetry was done in a N_2-saturated phosphate buffer (0.01 M) at pH 7.0 with a Yanako Model P-900 instrument. A three-electrode system (BAS Inc.) was used with a Pt auxiliary electrode and a glassy carbon working electrode against an Ag/AgCl (3 M NaCl) reference electrode. Electronic absorption spectra were recorded on a Shimadzu UV-240 spectrophotometer. The pHs of the solutions were measured on a Hitachi-Horiba F-14RS pH meter.

Results

Flash illumination of ZnPPMb (S. W. and H. H.) with a 30 μs pulse-width Xe lamp yielded about 70% triplet state of ZnPPMb (*ZnPPMb) on the basis of the absorbance change at 428 nm ($\Delta\varepsilon=\varepsilon(\text{ground})-\varepsilon(\text{triplet})=1.00\times10^5\ \text{M}^{-1}\,\text{cm}^{-1}$).[2)] Low concentrations of ZnPPMb were used at low concentraions of quenchers to maintain the pseudo-first order condition. The spontaneous decay rate constant agreed with the reference values (70—80 s^{-1}).[2—5)]

Quenching by $[Fe(CN)_6]^{3-}$. Similar kinetic results were obtained for both S. W. and H. H. *ZnPPMb. The quenching of *ZnPPMb by $[Fe(CN)_6]^{3-}$ obeys first-order kinetics for at least 85% of the reaction, and the observed first-order rate constant (k_{obsd}) was independent of the wavelengths monitored (428, 460, and 680 nm). Plots of k_{obsd} vs. the initial concentrations of $[Fe(CN)_6]^{3-}$ ($[\text{Fe(III)}]_0$) are shown in Fig. 1, indicating that the quenching rate increased with increasing $[\text{Fe(III)}]_0$ and that it reached a constant at higher $[\text{Fe(III)}]_0$. The saturation behavior of the quenching rate can be accounted for by Scheme 1, including the complex of $[Fe(CN)_6]^{3-}$ and ZnPPMb and/or *ZnPPMb. The rate law can be represented as

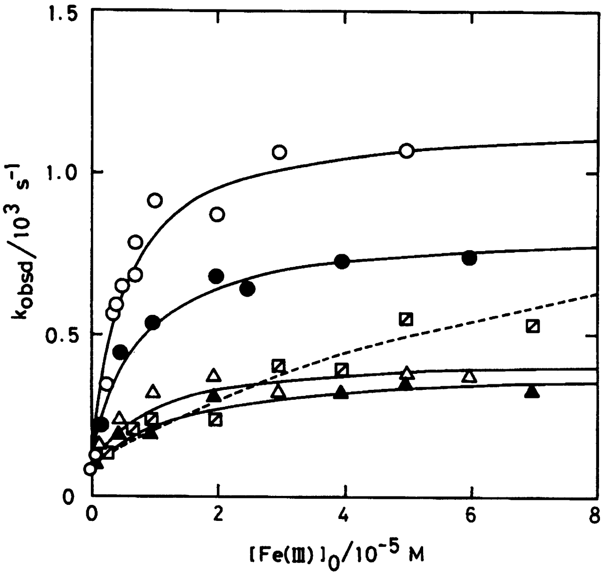
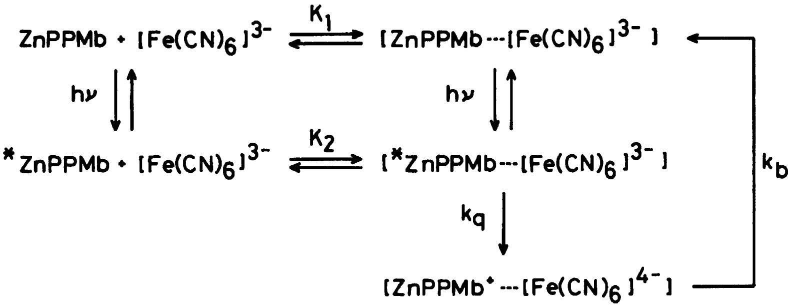


Fig. 1. Plots of k_{obsd} vs. the initial concentrations of $[Fe(CN)_6]^{3-}$ for the quenching of *ZnPPMb (S. W.) by $[Fe(CN)_6]^{3-}$ at 25 °C, $[\text{ZnPPMb}]_0=(0.3—5.0)\times10^{-6}$ M and pH 7.0 (0.01 M phosphate buffer). ○: $I=0.03$ M, ●: $I=0.10$ M, △: $I=0.20$ M, ▲: $I=0.30$ M, and ▨: $I=0.03$ M in the presence of 1.0×10^{-5} M poly-L-Lys.

$$-\frac{\mathrm{d}[{}^*\text{ZnPPMb}]}{\mathrm{d}t}=\left(k_0+\frac{k_{\text{q}}^{\text{intra}}K[\text{Fe(III)}]_0}{1+K[\text{Fe(III)}]_0}\right)[{}^*\text{ZnPPMb}], \tag{1}$$

where $k_{\text{obsd}}=k_0+k_{\text{q}}^{\text{intra}}K[\text{Fe(III)}]_0/(1+K[\text{Fe(III)}]_0)$ and k_0 is a spontaneous decay rate constant. Intermediates including ZnPPMb^+ could not be detected and the value of k_{obsd} was independent of the wavelengths. Therefore, the thermal backward ET reaction (k_b) may be faster than the quenching reaction. The values of $k_{\text{q}}^{\text{intra}}$ and K, the latter corresponding to K_1 and/or K_2, are listed in Table 1. Both $k_{\text{q}}^{\text{intra}}$ and K decrease with increasing ionic strength. The observed quenching rate constants tend to decrease slightly with increasing pH, arising from the decrease in the binding constant of the intracomplex.

Quenching by Cationic Quenchers. 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. One of the examples is shown in Fig. 2. The decay of *ZnPPMb monitored at 460 nm, where the absorption of *ZnPPMb is predominant, was monophasic and first order for at least 85% of the reaction. The values of k_{obsd} were linearly dependent on the concentrations of the quenchers (Figs. 3, 4, and 5). The slow phase reaction monitored at 680 nm was consistent with second-order kinetics (see Fig. 6) and the rate was independent of the concentrations of the quenchers. The second-order rate



Scheme 1.

Table 1. Rate Constants for the Intracomplex Quenching Reaction and Binding Constants for the Self-Associated Complex of *ZnPPMb and $[Fe(CN)_6]^{3-}$ at 25 °C and pH 7.0 (0.01 M Phosphate Buffer)

I/M	$k_q^{\mathrm{intra}}/10^3\ \mathrm{s}^{-1}$	$K/10^5\ \mathrm{M}^{-1}$
0.03	1.1±0.1	1.9±0.2
0.03[a)]	1.2±0.1	2.5±0.3
0.03[b)]	1.1±0.1	1.0±0.1
0.03[c)]	1.1±0.1	1.0±0.1
0.10	0.77±0.08	1.4±0.1
0.20	0.36±0.03	1.2±0.1
0.30	0.33±0.03	0.76±0.07

a) pH 6.5. b) pH 8.0. c) pH 9.3.

constants were evaluated from the latter linear portion of the plots. The absorption at 680 nm might be mainly due to a porphyrin radical cation ($ZnPPMb^+$). Therefore, the biphasic behavior, 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 *ZnPPMb with cationic quenchers can be represented as in Scheme 2. The rate constants for both photoinduced ET and thermal backward ET reactions (k_q and k_b) are listed in Table 2. The k_q values increase with increasing ionic strengths for all of the cationic quencher systems (see Figs. 3 and 7). The k_b values also increase with increasing ionic strengths.

The rate of the quenching reactions of *ZnPPMb by metmyoglobin and its imidazole adduct were slower than the spontaneous decay rate under these experimental conditions.

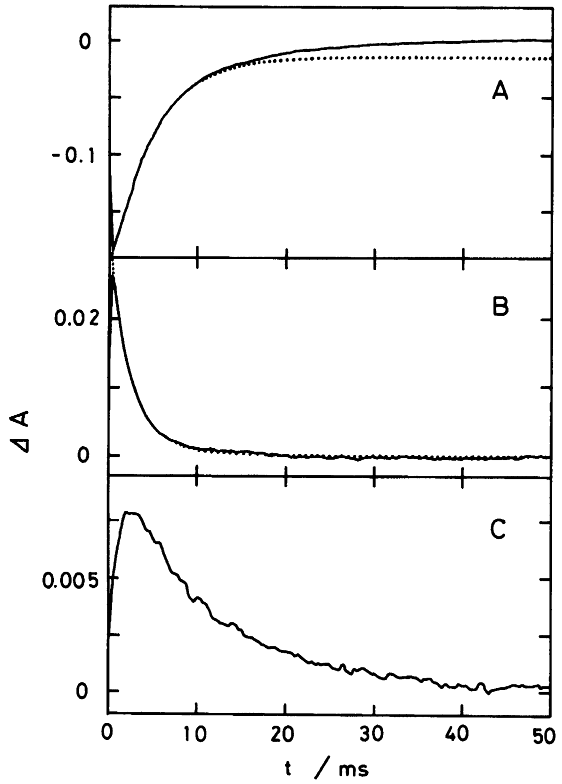


Fig. 2. Absorbance changes following production of *ZnPPMb (H. H.) by irradiation of ZnPPMb with a Xe-flash lamp in the presence of CMV^+ (1.2×10^{-5} M) at pH 7.0 (0.01 M phosphate buffer) and I=0.02 M. (A) 428 nm, (B) 460 nm, and (C) 680 nm. The dotted lines are of single-exponential fits for A and B.

Effects of Poly-L-Lys. Addition of a highly charged cation, poly-L-Lys (up to 2.0×10^{-5} M), into the 5.0×10^{-6} M ZnPPMb solution had no effect on the rate of spontaneous decay of *ZnPPMb. The effects of poly-L-Lys on the rate of quenching of *ZnPPMb by $[Fe(CN)_6]^{3-}$ and $[Ru(NH_3)_6]^{3+}$ ions are shown in Fig. 8. The quenching of *ZnPPMb by $[Fe(CN)_6]^{3-}$ was drastically inhibited by addition of poly-L-Lys. On the other hand, poly-L-Lys has only a small effect on the quenching rate of *ZnPPMb by $[Ru(NH_3)_6]^{3+}$, where the rate tends to increase with increasing concentrations of poly-L-Lys. The thermal backward ET reaction between $ZnPPMb^+$ and $[Ru(NH_3)_6]^{2+}$ was not affected by adding poly-L-Lys.

Effects of Poly-L-Glu. A highly charged anion, poly-L-Glu, has a small effect on the quenching rate of *ZnPPMb by both $[Fe(CN)_6]^{3-}$ and $[Ru(NH_3)_6]^{3+}$ ions; with increasing concentrations of poly-L-Glu, the former quencher decreased the rate of reaction, while the latter increased it (see Fig. 9). The rate of the thermal backward ET reaction between $ZnPPMb^+$ and $[Ru(NH_3)_6]^{2+}$ increased slightly with an increase in the concentrations of poly-L-Glu. Poly-L-Glu had no effect

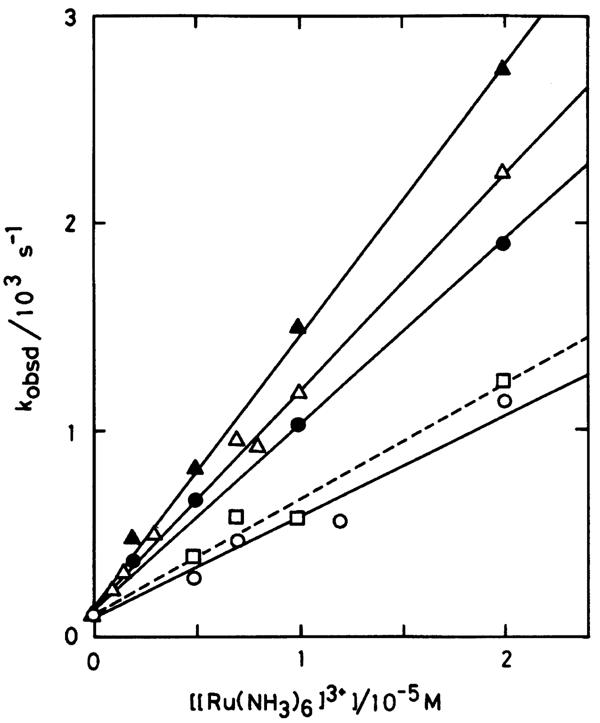


Fig. 3. Plots of k_{obsd} vs. the initial concentrations of $[Ru(NH_3)_6]^{3+}$ for the quenching of *ZnPPMb (S. W.) by $[Ru(NH_3)_6]^{3+}$ at 25 °C, $[ZnPPMb]_0=(0.3—5.0)\times10^{-6}$ M, and pH 7.0 (0.01 M phsophate buffer). ○: $I=0.02$ M, ●: $I=0.07$ M, △: $I=0.12$ M, ▲: $I=0.20$ M, and □: $I=0.02$ M for H. H. *ZnPPMb.

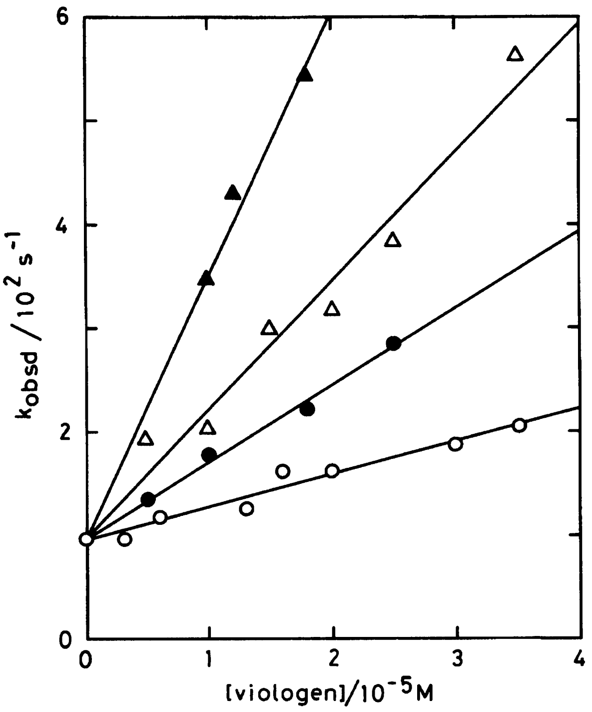


Fig. 4. Plots of k_{obsd} vs. the initial concentrations of viologens for the quenching of *ZnPPMb (H. H.) by viologens at 25 °C, $[ZnPPMb]_0=(0.3—5.0)\times10^{-6}$ M, pH 7.0 (0.01 M phosphate buffer), and $I=0.02$ M. ○: PTQ^{4+}, ●: BTQ^{4+}, △: ETQ^{4+}, and ▲: CMV^+.

on the rate of the spontaneous decay of *ZnPPMb as well as poly-L-Lys.

Effects of HMP. A negatively charged HMP (−6) in an aqueous neutral solution had a small effect on the spontaneous decay rate of *ZnPPMb; there was about 30% increase in the rate on addition of 2.0×10^{-5} M HMP. The effects of HMP on the quenching of *ZnPPMb were examined with PTQ^{4+} as a quencher. The observed quenching rate constants were linearly dependent on the concentrations of PTQ^{4+}, whether in the presence or absence of HMP (see Fig. 7). The quenching reaction was faster than that in the absence of HMP at low ionic strength, and there was no difference in the rate at high ionic strength. Figure 9 also shows the dependence of the concentrations of HMP on the quenching of *ZnPPMb by PTQ^{4+} at a constant concentration of PTQ^{4+}. The effects of HMP were small under the conditions shown in Fig. 9, but it is obvious that the rate of quenching becomes a maximum at [HMP]/[ZnPPMb]=2. The effects of HMP on the thermal backward ET reaction were similar to that for the quenching reaction.

Spectral Properties. Addition of $[Fe(CN)_6]^{3-}$ into the ZnPPMb solution did not appreciably modify the absorption spectrum of ZnPPMb in the visible region under the same conditions as those in the quenching measurements. The absorption spectrum of $[Fe(CN)_6]^{3-}$ in an aqueous solution at $I=0.02$ M changed on addition of poly-L-Lys with isosbestic points at 360 and 440 nm (Fig. 10). It is found that 1 mole of poly-L-Lys binds 3 to 4 moles of $[Fe(CN)_6]^{3-}$ ions from the plots of the change in absorbance at 419 nm vs. the ratio of $[\text{poly-L-Lys}]/[[Fe(CN)_6]^{3-}]$, although the change in absorbance was small. At a high ionic strength (0.5 M with NaCl), the spectrum of $[Fe(CN)_6]^{3-}$ did not change appreciably on addition of poly-L-Lys. Poly-L-Glu had little effect on the spectrum of $[Fe(CN)_6]^{3-}$ under the same conditions shown in Fig. 10.

The absorption spectra of $[Ru(NH_3)_6]^{3+}$ on addition of poly-L-Glu were measured in an aqueous solution against the same concentrations of poly-L-Glu solution as that of the additive. The absorbance increased over the range from 250 to 500 nm without any isosbestic points; the change in absorbance became constant at $[\text{poly-L-Glu}]/[[Ru(NH_3)_6]^{3+}]$=about 0.3—0.4 (Fig. 11). It is likely that poly-L-Glu interacts with $[Ru(NH_3)_6]^{3+}$ electrostatically to form an ion pair. On the other hand, ploy-L-Lys did not modify the spectrum of $[Ru(NH_3)_6]^{3+}$. When HMP was added into the solution of $[Ru(NH_3)_6]^{3+}$, even at 10^{-5} M level of concentration, precipitation occurred; it is suggested that a strong 2 : 1 complex forms between $[Ru(NH_3)_6]^{3+}$ and HMP.

The effects of HMP on the spectrum of PTQ^{4+} were examined in an aqueous solution at $I=0.02$ M. There were two isosbestic points at 240 and 280 nm in the

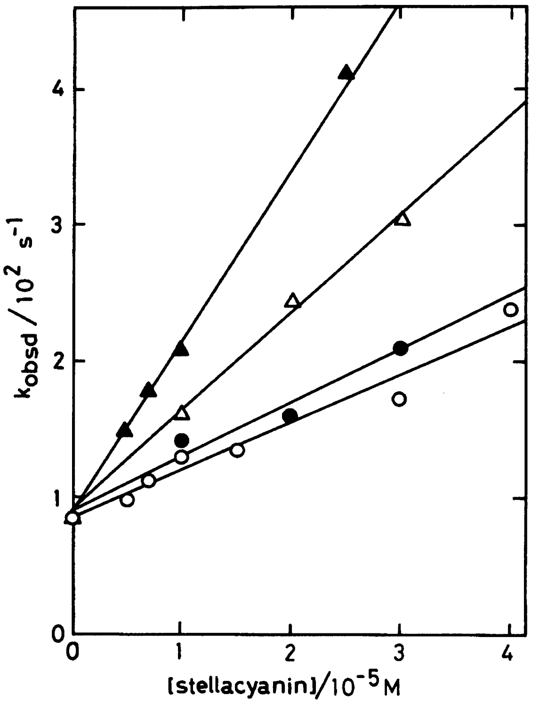


Fig. 5. Plots of $k_{\rm obsd}$ vs. the initial concentrations of stellacyanin for the quenching of *ZnPPMb (H. H.) by stellacyanin at 25 °C, $[\mathrm{ZnPPMb}]_0=(0.3—5.0)\times10^{-6}$ M, and pH 7.0 (0.01 M phosphate buffer). ○: $I=0.02$ M, ●: $I=0.09$ M, △: $I=0.20$ M, and ▲: $I=0.32$ M.

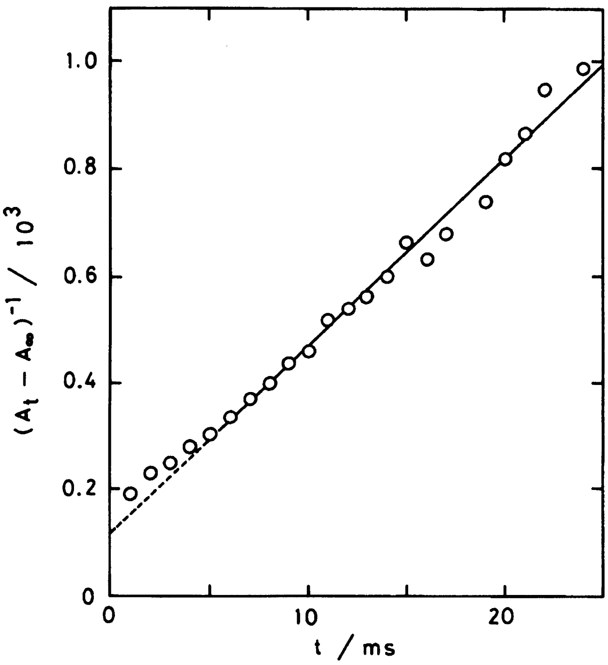
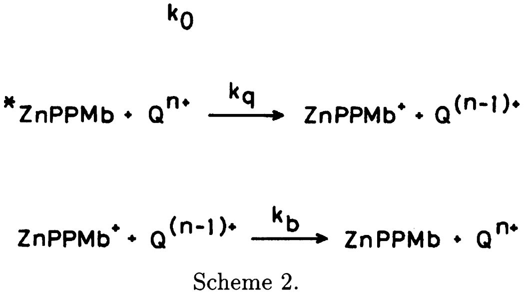


Fig. 6. Plots of $(A_t-A_\infty)^{-1}$ vs. t for the slow phase of the quenching of *ZnPPMb (S. W.) by $[\mathrm{Ru(NH_3)_6}]^{3+}$ at 25 °C, $[\mathrm{ZnPPMb}]_0=3.0\times10^{-6}$ M, $[\mathrm{Ru(III)}]_0=1.0\times10^{-5}$ M, pH 7.0 (0.01 M phosphate buffer), and $I=0.02$ M.



Scheme 2.

range from 200 to 400 nm on addition of HMP (Fig. 12). The absorbance became constant at $[\mathrm{HMP}]/[\mathrm{PTQ}^{4+}]=$ about 0.6, indicating a complex formation between PTQ^{4+} and HMP. Absorption spectral changes of PTQ^{4+} on addition of HMP in the presence of ZnPPMb were also measured, and there were two isosbestic points at the same wavelengths as in the absence of ZnPPMb. A larger amount of HMP was required for the absorbance to become constant than that in the absence of ZnPPMb ($[\mathrm{HMP}]/[\mathrm{PTQ}^{4+}]$=about 0.8), suggesting an interaction between ZnPPMb and HMP.

Cyclic Voltammetry. To confirm the interaction of cationic quenchers with poly-L-Lys, poly-L-Glu, and HMP, cyclic voltammograms were measured in a 0.01 M phosphate buffer at pH 7.0. The $[\mathrm{Ru(NH_3)_6}]^{3+}$ ion showed a quasi-reversible wave at $E_{1/2}=0.10$ V vs. NHE with a peak separation ($\Delta E_{\rm p}$) of 120 mV at a scan rate of 50 mV s^{-1}.[19] In the presence of poly-L-Glu the concentration of which is half of $[\mathrm{Ru(NH_3)_6}]^{3+}$, the cyclic voltammogram became reversible ($\Delta E_{\rm p}=60$ mV), and the $E_{1/2}$ value shifted to 0.0 V accompanying a decrease in peak currents by about 60% (Fig. 13). On the other hand, addition of poly-L-Lys ($[\text{poly-L-Lys}]/[[\mathrm{Ru(NH_3)_6}]^{3+}]=1.0$) had only a small effect on the values of $E_{1/2}$ (0.11 V) and $\Delta E_{\rm p}$ (105 mV). The cyclic voltammetry of PTQ^{4+} showed a quasi-reversible wave at $E_{1/2}=-0.25$ V vs. NHE with $\Delta E_{\rm p}=80$ mV at a scan rate of 50 mV s^{-1}.[21] On addition of HMP ($[\mathrm{HMP}]/[\mathrm{PTQ}^{4+}]=0.5$), the redox wave was separated into two redox cycles at -0.25 V ($\Delta E_{\rm p}=50$ mV) and -0.33 V ($\Delta E_{\rm p}=55$ mV), respectively.

Discussion

Intracomplex Quenching by $[\mathrm{Fe(CN)_6}]^{3-}$. The quenching of *ZnPPMb by $[\mathrm{Fe(CN)_6}]^{3-}$ occurs in a self-associated complex at high concentrations of $[\mathrm{Fe(CN)_6}]^{3-}$. It is known that $[\mathrm{Fe(CN)_6}]^{3-/4-}$ ions bind hemoproteins, such as cytochrome *c*,[22] and monomeric or tetrameric hemoglobin.[23,24] The binding constant of $[\mathrm{Fe(CN)_6}]^{3-}$ with myoglobin has not been reported, but it is suggested that the interaction is weaker in myoglobin than in cytochrome *c*, the binding constant of which is about 10^3 M^{-1};[22] it is therefore reasonable that the binding constant of myoglobin with $[\mathrm{Fe(CN)_6}]^{3-}$ is less than 10^3 M^{-1}. This estimated value

Table 2. Rate Constants for the Quenching and Thermal Backward ET Reactions of ZnPPMb at 25 °C and pH 7.0 (0.01 M Phosphate Buffer)

Quencher	I/M	k_q/M^{-1} s^{-1}	k_b/M^{-1} s^{-1}
Stellacyanin	0.02	$(3.5\pm0.2)\times10^6$	$(5.9\pm1.3)\times10^6$
	0.09	$(4.1\pm0.3)\times10^6$	$(6.7\pm0.9)\times10^6$
	0.20	$(7.5\pm0.4)\times10^6$	$(7.2\pm1.5)\times10^6$
	0.32	$(1.2\pm0.1)\times10^7$	$(8.6\pm1.5)\times10^6$
$[\mathrm{Ru(NH_3)_6}]^{3+}$	0.02	$(5.0\pm0.3)\times10^7$	$(2.6\pm0.3)\times10^7$
	0.07	$(9.0\pm0.5)\times10^7$	$(3.0\pm0.3)\times10^7$
	0.12	$(1.1\pm0.1)\times10^8$	$(3.6\pm0.4)\times10^7$
	0.20	$(1.3\pm0.1)\times10^8$	$(4.5\pm0.5)\times10^7$
$\mathrm{O_2}$	0.05	1.0×10^{8}[a]	
	0.20	1.3×10^{8}[b]	
$\mathrm{ETQ^{4+}}$	0.02	$(1.3\pm0.1)\times10^7$	$(3.3\pm0.5)\times10^7$
	0.05	$(2.1\pm0.1)\times10^7$	$(4.5\pm0.5)\times10^7$
	0.09	$(3.0\pm0.2)\times10^7$	$(7.3\pm0.7)\times10^7$
	0.20	$(4.7\pm0.3)\times10^7$	$(8.0\pm0.7)\times10^7$
	0.30	$(6.1\pm0.4)\times10^7$	$(9.7\pm0.7)\times10^7$
$\mathrm{PTQ^{4+}}$	0.02	$(3.2\pm0.2)\times10^6$	$(2.4\pm0.6)\times10^7$
	0.12	$(2.2\pm0.2)\times10^7$	$(5.3\pm0.5)\times10^7$
	0.30	$(4.1\pm0.3)\times10^7$	$(6.9\pm0.7)\times10^7$
$\mathrm{AQS^-}$	0.05	$(2.9\pm0.3)\times10^{8}$[a]	
$\mathrm{BTQ^{4+}}$	0.02	$(7.5\pm0.6)\times10^6$	$(2.4\pm0.8)\times10^7$
$\mathrm{CMV^+}$	0.02	$(2.6\pm0.2)\times10^7$	$(1.1\pm0.2)\times10^8$
$\mathrm{MV^{2+}}$	0.02	$(4.7\pm0.5)\times10^7$	$(4.0\pm1.0)\times10^7$
	0.02	2.7×10^{7}[c]	
	0.05	$(4.5\pm0.4)\times10^{7}$[a]	

a) Ref. 3. b) Ref. 2. c) For horse skeletal muscle ZnPPMb. Ref. 6.

is much smaller than that of the binding constant obtained in the quenching reaction of *ZnPPMb by $[\mathrm{Fe(CN)_6}]^{3-}$ ($K\approx10^5$ M^{-1}). Therefore, a self-associated complex might form between the excited state of ZnPPMb and $[\mathrm{Fe(CN)_6}]^{3-}$, i. e. $K\approx K_2$. Both $k_\mathrm{q}^\mathrm{intra}$ and K decrease with increasing ionic strength adjusted with NaCl. The $\mathrm{Na^+}$ and $\mathrm{Cl^-}$ ions can interact with $[\mathrm{Fe(CN)_6}]^{3-}$ and myoglobin, respectively; therefore, the complex of *ZnPPMb and $[\mathrm{Fe(CN)_6}]^{3-}$ may be detached by them. Decrease in $k_\mathrm{q}^\mathrm{intra}$ with increasing ionic strengths is attributable to an increase in the separation distance of the redox partners in the self-associated complex with the entrance of $\mathrm{Na^+}$ and $\mathrm{Cl^-}$ ions. The value of $k_\mathrm{q}^\mathrm{intra}$ is almost independent of pH and that of K decreases with increasing pHs. The positively charged amino acid residues in myoglobin are Arg and Lys, the latter being predominant on the surface of the protein. The isoelectric point of ZnPPMb has been estimated to be 7.4 by Cowan and Gray.[25] The decrease in K values with increasing pHs is probably due to weakening of the interaction of $[\mathrm{Fe(CN)_6}]^{3-}$ with Lys residues by deprotonation of amino acid residues. Insensitivity of $k_\mathrm{q}^\mathrm{intra}$ to pH changes suggests that the binding site of ZnPPMb with $[\mathrm{Fe(CN)_6}]^{3-}$ is not altered over the pH range from 6.5 to 9.3.

To confirm the electrostatic interaction of *ZnPPMb with $[\mathrm{Fe(CN)_6}]^{3-}$, the effects of highly charged polyamino acids, poly-L-Lys and poly-L-Glu, on the rate of quenching were examined at a low ionic strength. Both poly-L-Lys and poly-L-Glu are randomly coiled at neutral pH.[26] Addition of poly-L-Lys drastically inhibited the quenching of *ZnPPMb, which tends to reach the spontaneous decay rate of *ZnPPMb at high concentrations of poly-L-Lys (see Fig. 8). A slight decrease in the rate of the quenching by $[\mathrm{Fe(CN)_6}]^{3-}$ in the presence of anionic polyamino acid, poly-L-Glu, arises from increasing ionic strengths on addition of poly-L-Glu into a 0.015 M phosphate buffer solution at pH 7.0: I=0.063 M for 2.0×10^{-5} M poly-L-Glu (a mean charge of −79). The effects of poly-L-Lys are not due to ionic strength, because the ionic strength of a 2.0×10^{-5} M poly-L-Lys solution is only 3.4×10^{-3} M (a mean charge of poly-L-Lys is +18). Moreover, both poly-L-Lys and poly-L-Glu had no effect on the spontaneous decay rate of *ZnPPMb, suggesting that the interaction of *ZnPPMb with them is not as strong as that with $[\mathrm{Fe(CN)_6}]^{3-}$. The effects of poly-L-Lys can therefore be rationalized by the fact that poly-L-Lys takes $[\mathrm{Fe(CN)_6}]^{3-}$ ions away from *ZnPPMb to keep it at a long distance from the protein. Absorption spectral measurements support such a strong interaction of poly-L-Lys and $[\mathrm{Fe(CN)_6}]^{3-}$ (see Fig. 10). The intracomplex ET quenching reaction of *ZnPPMb with $[\mathrm{Fe(CN)_6}]^{3-}$ can be controlled by adjusting ionic strengths with inorganic salts and by adding a highly charged cation, such as poly-L-Lys.

Intermolecular Quenching by Cationic Quen-

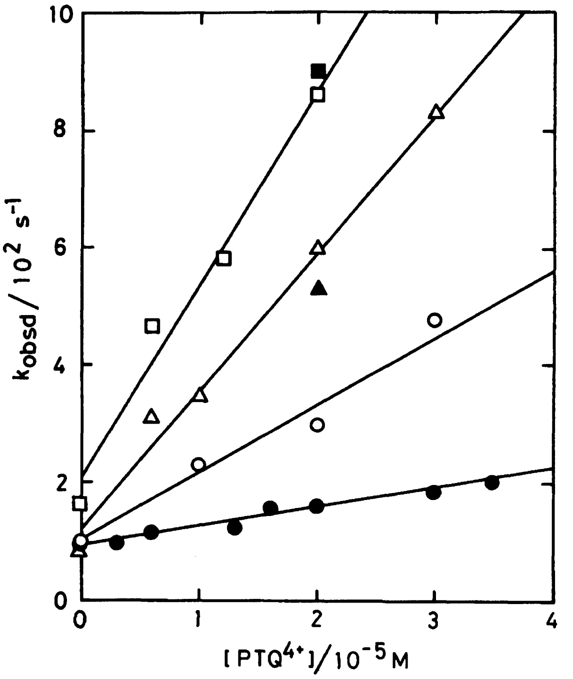


Fig. 7. Plots of k_{obsd} vs. the initial concentrations of PTQ^{4+} for the quenching of *ZnPPMb (H. H.) by PTQ^{4+} in the presence (1.0×10^{-5} M, open symbols) or absence (closed symbols) of HMP at 25 °C, $[ZnPPMb]_0=(1.0—5.0)\times10^{-6}$ M, and pH 7.0 (0.01 M phosphate buffer). ○, ●: $I=0.02$ M, △, ▲: $I=0.12$ M, and □, ■: $I=0.30$ M.

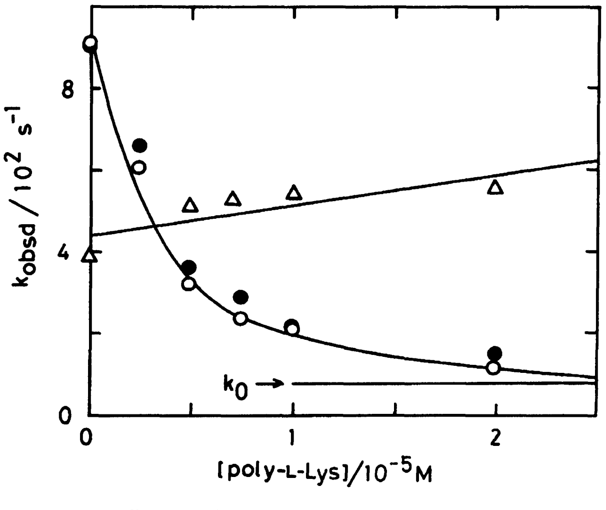


Fig. 8. Effects of poly-L-Lys on the quenching of *ZnPPMb (S. W.) by $[Fe(CN)_6]^{3-}$ and $[Ru(NH_3)_6]^{3+}$ at 25 °C, $[ZnPPMb]_0=(0.3—5.0)\times10^{-6}$ M, and pH 7.0 (0.01 M phosphate buffer). ○: $[Fe(CN)_6]^{3-}$ (1.0×10^{-5} M), ●: $[Fe(CN)_6]^{3-}$ (1.0×10^{-5} M) for H. H. myoglobin, and △: $[Ru(NH_3)_6]^{3+}$ (5.0×10^{-6} M).

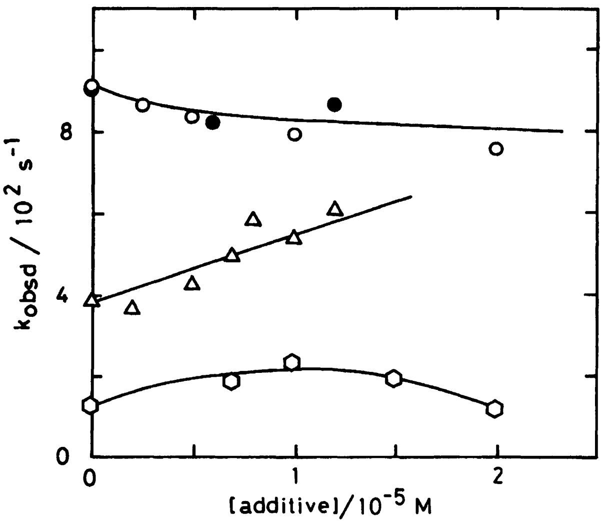


Fig. 9. Effects of poly-L-Glu and HMP on the quenching of *ZnPPMb (S. W.) by $[Fe(CN)_6]^{3-}$, $[Ru(NH_3)_6]^{3+}$, and PTQ^{4+} at 25 °C, $[ZnPPMb]_0=(0.3—3.0)\times10^{-6}$ M, and pH 7.0 (0.01 M phosphate buffer). ○: $[Fe(CN)_6]^{3-}$ (1.0×10^{-5} M) for S. W. myoglobin and additive=poly-L-Glu, ●: $[Fe(CN)_6]^{3-}$ (1.0×10^{-5} M) for H. H. myoglobin and additive=poly-L-Glu, △: $[Ru(NH_3)_6]^{3+}$ (5.0×10^{-6} M) for S. W. myoglobin and additive=poly-L-Glu, and ⬡: PTQ^{4+} (1.0×10^{-5} M) for H. H. myoglobin and additive=HMP.

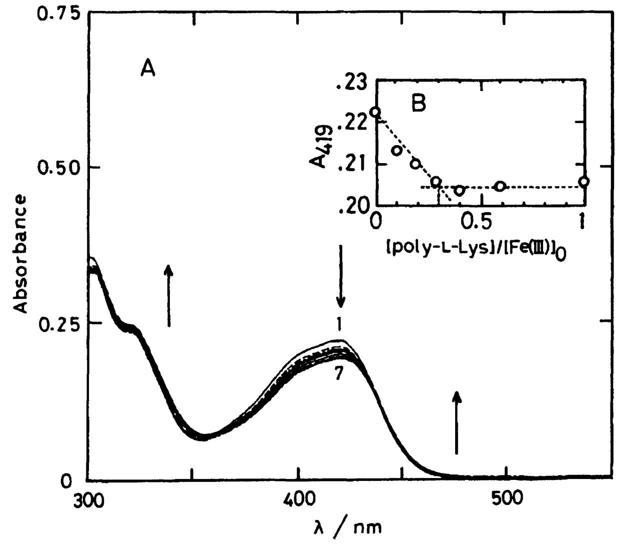


Fig. 10. A) Absorption spectral changes of $[Fe(CN)_6]^{3-}$ (2.0×10^{-4} M) on addition of poly-L-Lys at 25 °C and pH 7.0 (10 mM phosphate buffer). (1) in the absence of poly-L-Lys, (2) 2.0×10^{-5} M, (3) 4.0×10^{-5} M, (4) 6.0×10^{-5} M, (5) 8.0×10^{-5} M, (6) 1.2×10^{-4} M, and (7) 2.0×10^{-4} M. B) Plots of the absorbance at 419 nm vs. [poly-L-Lys]/[Fe(III)]$_0$.

chers. None of the cationic quenchers examined in this work formed a self-associated complex with *ZnPPMb, and intermolecular quenching occurred. The ionic strength dependence on the quenching rate suggests that the reactive site of *ZnPPMb is a positively charged residue: k_q increases with increasing ionic strengths. Plots of log k_q vs. $I^{1/2}$ were linear and the estimated charge of *ZnPPMb was +0.45 for [Ru-

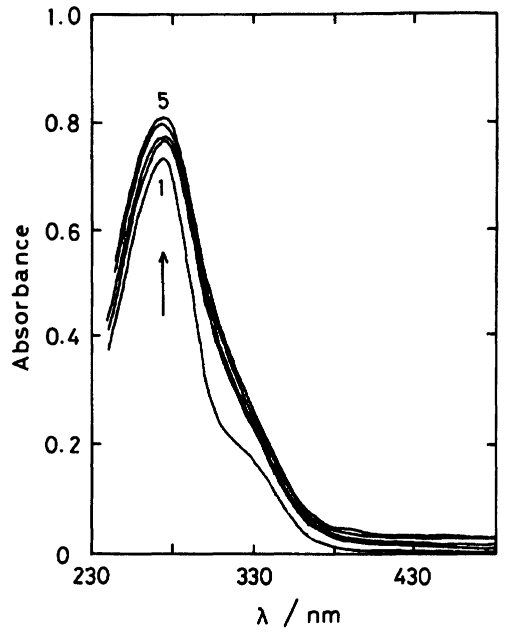


Fig. 11. Difference absorption spectra of [Ru$(NH_3)_6]^{3+}$ (2.0×10^{-5} M) in the presence of poly-L-Glu at 25 °C and pH 7.0 (0.01 M phosphate buffer). The poly-L-Glu solution was used as a reference. (1) in the absence of poly-L-Glu, (2) 2.0×10^{-6} M, (3) 4.0×10^{-6} M, (4) 6.0×10^{-6} M, and (5) 8.0×10^{-6} M.

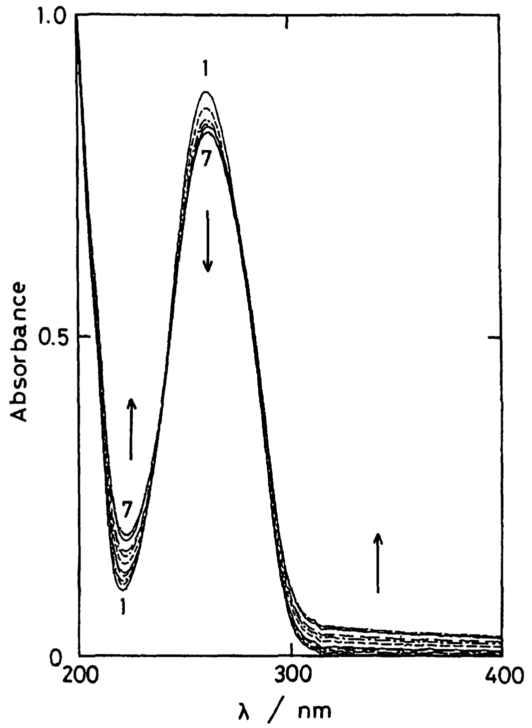


Fig. 12. Absorption spectral changes of PTQ^{4+} (2.0×10^{-5} M) on addition of HMP at 25 °C and pH 7.0 (0.01 M phosphate buffer). (1) in the absence of HMP, (2) 2.0×10^{-6} M, (3) 4.0×10^{-6} M, (4) 6.0×10^{-6} M, (5) 8.0×10^{-6} M, (6) 1.0×10^{-5} M, and (7) 1.2×10^{-5} M.

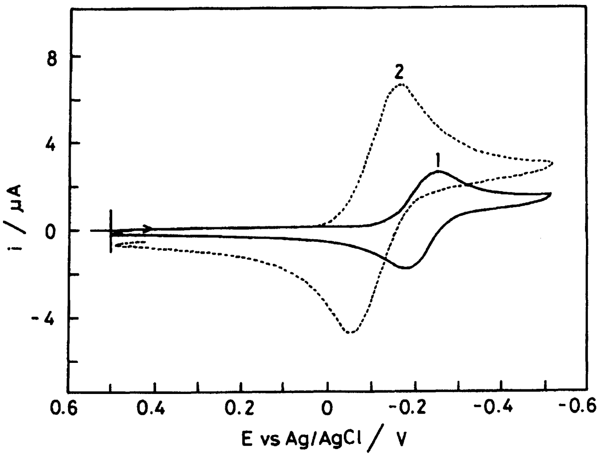


Fig. 13. Cyclic voltammograms of $[Ru(NH_3)_6]^{3+}$ (7.0×10^{-4} M) in the presence and absence of poly-L-Glu at 25 °C, pH 7.0 (0.01 M phosphate buffer), and a scan rate of 50 mV s^{-1}. (1) in the presence of poly-L-Glu and (2) in the absence of poly-L-Glu (3.6×10^{-4} M).

$(NH_3)_6]^{3+}$, +0.38 for ETQ^{4+}, and +0.63 for PTQ^{4+}. The estimated charge of +0.5 (mean) suggests that the reactive site of *ZnPPMb 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 highly charged cation, 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. On the other hand, we obtained evidence for complex formation between $[Ru(NH_3)_6]^{3+}$ and poly-L-Glu. A negative shift in the $E_{1/2}$ value on addition of poly-L-Glu might arise from a decrease in the effective charge of $[Ru(NH_3)_6]^{3+}$ by complex formation. However, poly-L-Glu had only a small effect on the quenching rate of *ZnPPMb by [Ru$(NH_3)_6]^{3+}$. The expected acceleration of the quenching rate due to a decrease in charge repulsion between *ZnPPMb and $[Ru(NH_3)_6]^{3+}$ in the presence of poly-L-Glu may be canceled by an increase in the separation distance between the redox partners through the complex formation of $[Ru(NH_3)_6]^{3+}$ and poly-L-Glu.

Sodium *cyclo*-hexaphosphate is a cyclic hexamer of phosphate with −6 charge, and the maximum distance between peripheral oxygen atoms is 0.87 nm.[27] Spectral and cyclic voltammetry measurements showed complex formation between PTQ^{4+} and HMP. The rate of quenching of *ZnPPMb by PTQ^{4+} in the presence of HMP became faster than that in the absence of HMP at low ionic strengths. At high ionic strengths there was no difference in the rate between the two. Acceleration of the quenching rate is attributable to the decrease in charge repulsion between *ZnPPMb and PTQ^{4+} by the formation of the complex of PTQ^{4+} with HMP. From

Table 3. Estimated Self-Exchange Rate Constants for $ZnPPMb^{+/*}$ and $ZnPPMb^{+/0}$ Systems Based on the Marcus Theory[a)]

Quencher	E°	k_{ex} for $ZnPPMb^{+/*}$	Reductant	E°	k_{ex} for $ZnPPMb^{+/0}$
	V	$M^{-1}\,s^{-1}$		V	$M^{-1}\,s^{-1}$
Stellacyanin[b)]	0.18[c)]	3.4×10^{-6}	Stellacyanin	0.18	1.8×10^{-3}
$[Ru(NH_3)_6]^{3+}$ [d)]	0.05[e)]	1.8	$[Ru(NH_3)_6]^{2+}$	0.05	4.1×10^{-2}
O_2[f)]	−0.16[g)]	5.6×10^{4}	PTQ^{2+} [h)]	−0.19[i)]	4.0×10^{-7}
ETQ^{4+} [j)]	−0.28[i)]	4.9×10^{-2}	ETQ^{2+} [j)]	−0.28[i)]	3.5×10^{-10}
PTQ^{4+} [j)]	−0.33[i)]	1.1×10^{-2}	BTQ^{2+} [k)]	−0.29[i)]	2.4×10^{-9}
AQS^{-} [l)]	−0.38[m)]	9.0×10^{2}	CMV	−0.42	2.2×10^{-10}
BTQ^{4+} [j)]	−0.39[i)]	4.6×10^{-1}	MV^{+}	−0.45	8.6×10^{-12}
CMV^{+} [j)]	−0.42[n)]	1.6×10			
MV^{2+} [j)]	−0.45[i)]	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 $\ln f_{12}=(\ln K_{12})^2/4\ln(k_{11}k_{22}/10^{22})$. Ref. 30. b) $k_{22}=1.2\times10^5\ M^{-1}\,s^{-1}$. Ref. 31. c) Ref. 14. d) $k_{22}=4\times10^3\ M^{-1}\,s^{-1}$. Ref. 16. e) Ref. 20. f) $k_{22}=4.5\times10^2\ M^{-1}\,s^{-1}$. Ref. 32. g) Ref. 33. h) $k_{22}=4.6\times10^5\ M^{-1}\,s^{-1}$. Ref. 18. i) Ref. 18. j) $k_{22}=1\times10^8\ M^{-1}\,s^{-1}$. Refs. 18 and 34. k) $k_{22}=5.1\times10^6\ M^{-1}\,s^{-1}$. Ref. 18. l) $k_{22}=1\times10^8\ M^{-1}\,s^{-1}$. Ref. 35. m) Ref. 36. n) Ref. 37.

the ionic strength dependence on k_q in the presence of HMP, the effective charge of PTQ^{4+} decreased to +2.2, being a reasonable value on the basis of the spectral titration of PTQ^{4+} with HMP; a 2 : 1 complex of PTQ^{4+} with HMP may form under these experimental conditions. Since such complex formation is not important at high ionic strengths, there would be little effect of HMP.

Stellacyanin is a blue copper glycoprotein with 107 amino acids and the net charge of the protein is positive at a neutral pH ($pI=9.9$).[28)] The rate of quenching of *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 *ZnPPMb is +0.5. A three-dimensional structure of stellacyanin has been proposed on the basis of a calculated model.[29)] Wherland et al.[29a)] have discussed the reactive site of stellacyanin for the reduction by Cr(II) which is most probably bound to Asp 49. They suggested that Tyr 85, being adjacent to Asp 49 and Lys 50, is involved in the redox reactions of stellacyanin along with the partially exposed His 92. The former residue is 1.0 nm from the Cu(II) center and near a positive patch (Lys 54, 83, and 98); therefore, the quenching of *ZnPPMb by stellacyanin may occur between the heme pocket of *ZnPPMb and the same region of stellacyanin as that for the Cr(II) reduction.

Thermal Backward ET Reaction. Aono et al.[6)] have observed a methylviologen radical cation in the quenching of *ZnPPMb by MV^{2+}. However, they have not followed the subsequent thermal backward ET reaction. We observed the thermal backward ET process for all of the cationic quencher systems examined in this work including MV^{2+}, which was consistent with the second-order kinetics. The k_b values increase with increasing ionic strengths, indicating the reaction between cationic species. Cationic poly-L-Lys had no effect on the thermal backward ET reaction. On the other hand, anionic poly-L-Glu and HMP had an effect similar to that for the quenching reaction: poly-L-Glu or HMP increased the k_b values at low ionic strengths and had no effect on the rate at high ionic strengths. This arises from the fact that the reaction occurs between a positive site of $ZnPPMb^+$ and the radical cation of the quencher.

In the reactions of bis(viologens), ETQ^{4+}, PTQ^{4+}, and BTQ^{4+}, a disproportionation reaction of the monoradical cation ($Q\text{-}Q^{3+}$) is much faster than the backward ET reaction.[18)] Therefore, the backward ET reaction must correspond to that of the diradical cation ($Q\text{-}Q^{2+}$):

$$2Q\text{-}Q^{3+} \longrightarrow Q\text{-}Q^{2+} + Q\text{-}Q^{4+} \quad \text{fast} \tag{2}$$

$$ZnPPMb^{+} + Q\text{-}Q^{2+} \xrightarrow{k_b} ZnPPMb + Q\text{-}Q^{3+} \tag{3}$$

Mechanism of Reaction. Both k_q and k_b values listed in Table 1 along with those from the literature are in a narrow range for a variety of quenchers and are smaller than the diffusion-controlled limit. Application of the Marcus theory is unsuccessful for these systems. 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).[38)] The disagreement in the values of the estimated self-exchange rate constants for the $ZnPPMb^{+/0}$ system is more serious. 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. 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} means that the quenching of *ZnPPMb is not controlled by the ET step. Barboy and Feitelson[3,4] have proposed a conformational gating mechanism for the bimolecular quenching reaction of *ZnPPMb. They have found similar activation energies for the quenching by O_2, MV^{2+}, and AQS^- (5.8—7.4 kcal mol^{-1}, 1 cal = 4.184 J). They also suggest 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 *ZnPPMb or ZnPPMb$^+$. We propose the following mechanism where both *ZnPPMb and ZnPPMb$^+$ have two conformers, active and inactive forms:

$$\mathrm{ZnPPMb} \underset{k_0}{\overset{h\nu}{\rightleftarrows}} {}^*(\mathrm{ZnPPMb})_\mathrm{A} \tag{4}$$

$$^*(\mathrm{ZnPPMb})_\mathrm{A} + \mathrm{Q}^{n+} \rightleftarrows {}^*(\mathrm{ZnPPMb})_\mathrm{B} + \mathrm{Q}^{n+} \tag{5}$$

$$^*(\mathrm{ZnPPMb})_\mathrm{B} + \mathrm{Q}^{n+} \xrightarrow{\mathrm{ET}} (\mathrm{ZnPPMb}^+)_\mathrm{B} + \mathrm{Q}^{(n-1)+} \tag{6}$$

$$(\mathrm{ZnPPMb}^+)_\mathrm{B} + \mathrm{Q}^{(n-1)+} \rightleftarrows (\mathrm{ZnPPMb}^+)_\mathrm{A} + \mathrm{Q}^{(n-1)+} \tag{7}$$

$$(\mathrm{ZnPPMb}^+)_\mathrm{A} + \mathrm{Q}^{(n-1)+} \xrightarrow{k_\mathrm{b}} \mathrm{ZnPPMb} + \mathrm{Q}^{n+} \tag{8}$$

After excitation, *ZnPPMb which is referred to as 'form A' converts to another active 'form B' (Eq. 5). The quenching rate is dependent on the concentrations of quenchers. Therefore, the formation of the active form B is induced by attacking of Q^{n+}. Then the ET quenching occurs in Eq. 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 and its imidazole adduct, the latter being much more reactive than the former upon reduction,[39] had no effect on the spontaneous decay of *ZnPPMb. Therefore, metmyoglobin might not induce any conformational changes in *ZnPPMb. Although no direct evidence has been reported for the conformational change of ZnPPMb induced by small molecules, the work on the autoxidation of oxymyoglobin induced by detergents has demonstrated that the conformational change of oxymyoglobin occurs in the ms time scale.[40]

This research was partly supported by a Grant-in-Aid for Scientific Research Nos. 03453049 (to K. T.) and 04225211 (to T. S.) from the Ministry of Education, Science and Culture, and by a grant from Itoh Science Foundation (to K. T.).

References

1) a) R. A. Marcus and N. Sutin, *Biochim. Biophys. Acta*, **811**, 265 (1985); b) S. L. Mayo, W. R. Ellis, Jr., R. J. Crutchley, and H. B. Gray, *Science*, **233**, 948 (1986); c) G. McLendon, *Acc. Chem. Res.*, **21**, 160 (1988).

2) H. Zemel and B. M. Hoffman, *J. Am. Chem. Soc.*, **103**, 1192 (1981).

3) N. Barboy and J. Feitelson, *Biochemistry*, **26**, 3240 (1987).

4) N. Barboy and J. Feitelson, *Biochemistry*, **28**, 5450 (1989).

5) A. C. Shosheva, P. K. Christova, and B. P. Atanasov, *Biochim. Biophys. Acta*, **957**, 202 (1988).

6) S. Aono, S. Nemoto, and I. Okura, *Bull. Chem. Soc. Jpn.*, **65**, 591 (1992).

7) B. M. Hoffman and M. A. Ratner, *J. Am. Chem. Soc.*, **109**, 6237 (1987).

8) G. McLendon, K. Pardue, and P. Bak, *J. Am. Chem. Soc.*, **109**, 7540 (1987).

9) J. Feitelson and G. McLendon, *Biochemistry*, **30**, 5051 (1991).

10) a) K. Tsukahara and S. Asami, *Chem. Lett.*, **1991**, 1337; b) K. Tsukahara and M. Okada, *Chem. Lett.*, **1992**, 1543.

11) a) K. Tsukahara and Y. Yamamoto, *J. Biochem. (Tokyo)*, **93**, 15 (1983); b) K. Tsukahara, *Inorg. Chim. Acta*, **124**, 199 (1986).

12) a) F. W. J. Teale, *Biochim. Biophys. Acta*, **35**, 543 (1959); b) W. S. Caughey, W. Y. Fujimoto, A. J. Bearden, and T. H. Moss, *Biochemistry*, **5**, 1255 (1966); c) K. Tsukahara, T. Okazawa, H. Takahashi, and Y. Yamamoto, *Inorg. Chem.*, **25**, 4756 (1986).

13) B. Reinhammar, *Biochim. Biophys. Acta*, **205**, 35 (1970).

14) T. Sakurai, O. Ikeda, and S. Suzuki, *Inorg. Chem.*, **29**, 4715 (1990).

15) B. G. Malmstrom, B. Reinhammar, and T. Vanngard, *Biochim. Biophys. Acta*, **205**, 48 (1970).

16) T. J. Meyer and H. Taube, *Inorg. Chem.*, **7**, 2369 (1968).

17) a) M. Furue and S. Nozakura, *Chem. Lett.*, **1980**, 821; b) M. Furue and S. Nozakura, *Bull. Chem. Soc. Jpn.*, **55**, 513 (1982).

18) S. J. Atherton, K. Tsukahara, and R. G. Wilkins, *J. Am. Chem. Soc.*, **108**, 3380 (1986).

19) A reversible redox couple has been reported in an aqueous 0.1 M $NaBF_4$ solution and a positive shift in potentials was observed at higher ionic strengths. See Refs. 16 and 20.

20) H. S. Lim, D. J. Barclay, and F. C. Anson, *Inorg. Chem.*, **11**, 1460 (1972).

21) A reversible $2e^-$-redox wave has been observed at −0.26 V vs. NHE using a Pt working electrode. See Ref. 18.

22) E. Stellwagen and R. D. Cass, *J. Biol. Chem.*, **250**, 2095 (1975).

23) A. W. Addison and S. Burman, *Biochim. Biophys. Acta*, **828**, 362 (1985).

24) B. M. Hoffman and C. Bull, *Proc. Natl. Acad. Sci. U. S. A.*, **73**, 800 (1976).

25) J. A. Cowan and H. B. Gray, *Inorg. Chem.*, **28**, 2074 (1989).

26) M. Nagasawa and A. Holtzer, *J. Am. Chem. Soc.*, **86**, 538 (1964).

27) A. McAdam, K. H. Jost, and B. Beasley, *Acta Crys-*

tallogr., Sect. B, **B28**, 2740 (1972).

28) C. Bergman, E.-K. Gandvik, P. O. Nyman, and L. Strid, *Biochem. Biophys. Res. Commun.*, **77**, 1052 (1977).

29) a) S. Wherland, O. Farver, and I. Pecht, *J. Mol. Biol.*, **204**, 407 (1988); b) B. A. Fields, J. M. Guss, and H. C. Freeman, *J. Mol. Biol.*, **222**, 1053 (1991).

30) R. A. Marcus, *Annu. Rev. Phys. Chem.*, **15**, 155 (1964).

31) S. Dahlin, B. Reinhammer, and M. T. Wilson, *Biochem. J.*, **218**, 609 (1984).

32) J. Lind, X. Shen, G. Merenyi, and B. O. Jonsson, *J. Am. Chem. Soc.*, **111**, 7654 (1989).

33) D. T. Sawyer and J. S. Valentine, *Acc. Chem. Res.*, **14**, 393 (1981).

34) K. Tsukahara and R. G. Wilkins, *J. Am. Chem. Soc.*, **107**, 2632 (1985).

35) D. Meisel and R. W. Fessenden, *J. Am. Chem. Soc.*, **98**, 7505 (1976).

36) D. Meisel and P. Neta, *J. Am. Chem. Soc.*, **97**, 5198 (1975).

37) K. Tsukahara and H. Todorobaru, *Chem. Lett.*, **1992**, 1181.

38) Although the electrostatic interactions are important at low ionic strengths, we ignored the work terms in the calculation. The discrepancy in the estimated self-exchange rate constants cannot be explained by only the work terms.

39) Z. Bradić, K. Tsukahara, P. C. Wilkins, and R. G. Wilkins, "Frontiers in Bioinorganic Chemistry," ed by A. V. Xavier, VCH Publishers, Weinheim, Germany (1986), p. 336.

40) K. Kasuga, S. Nakao, T. Kamezawa, K. Tsukahara, and Y. Yamamoto, *Biochim. Biophys. Acta*, **788**, 313 (1984).