Stereoselective Photoinduced Electron-Transfer Reactions of Zinc Myoglobin with Optically Active Viologens†

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Photoinduced electron-transfer reactions between zinc-substituted myoglobin and optically active viologens and bisviologens, containing ((naphthyl-, ((phenyl-, and ((cyclohexyl)ethyl)carbamoyl)methyl groups, have been studied at 25 °C, pH 7.0 (a 0.01 M phosphate buffer), and various ionic strengths. The excited triplet state of zinc myoglobin was preferentially quenched by (*S*,*S*)-isomers of optically active viologens; both ratios of the quenching rate constants and back-electron-transfer rate constants, $k((S,S))/k((R,R))$, range from 1.1 to 1.5 at an ionic strength of 0.02 M with the order naphthyl > phenyl ≥ cyclohexyl for the substituents. Stereoselectivity decreased with increasing ionic strengths. The steric bulk of the substituents of viologen may induce the conformational change of zinc myoglobin more effectively due to the steric repulsion between naphthyl groups and the polypeptide chain of zinc myoglobin.

Introduction

Photoinduced electron-transfer (ET) reactions of metalloproteins have received considerable attention in the fields of both chemistry and biochemistry. In the case of zinc-substituted hemoproteins, the excited triplet state is bimolecularly quenched by a variety of quenchers and several mechanisms, including the conformational gating mechanism,[1] have been suggested for zinc cytochrome *c*,[2] hemoglobin,[3] and myoglobin.[4,5] We have recently proposed that the conformational change of the excited triplet state of zinc myoglobin (^{3}(ZnMb)*) to an active form is induced by attacking of external quenchers and that the charge and the steric bulk of the quenchers play an important role in the quenching process.[5] As chirality is an obvious property of reactive sites in proteins, optically active substances must discriminate the environment around the reactive site at the surface of metalloproteins. Stereoselectivity in the ET reactions between metalloproteins and chiral metal complexes has been recently reported by using spinach plastocyanin,[6] horse cytochrome *c*,[7] and plant ferredoxin.[8] There is, however, no report on the stereoselectivity in the reactions between metalloprotein and a chiral organic reducing agent, especially in the photoinduced ET reaction. Rau and Ratz[9] have reported the stereoselectivity in the luminescence quenching of chiral ^{3}([Ru(bpy)$_3$]$^{2+}$)* by optically active viologen, 1-methyl-1′-[(3*S*)-(−)-3-pinanylmethyl]-4,4′-bipyridinium dichloride. Viologens are diquaternary salts of 4,4′-bipyridine and have been used extensively as mediators in catalytic photolysis of water under visible light with a sensitizer.[10] They undergo effective one-electron redox reactions with hemoproteins and excited triplet state of zinc-substituted hemoproteins.[3−5,11,12] Therefore, we chose the optically active viologens and bisviologens (**1**−**13**) as quenchers (Chart 1), which were newly synthesized, to investigate the ET quenching and back ET reactions of ^{3}(ZnMb)*. The preliminary communication has been reported elsewhere.[13]

Experimental Section

Reagents. Metmyoglobin from horse heart muscle (Sigma) was purified as previously described.[14] Recombination of (protoporphyrinIXato(2−))zinc(II) (Sigma) with apomyoglobin[15] was carried out at 4 °C in the dark using a method that has been published.[5] The concentrations of ZnMb were determined spectrophotometrically (ϵ_{428} = 1.53 × 10^5 M^{-1} cm^{-1}).[16] The ZnMb solution, whose absorption ratio of A_{428}/A_{280} is greater than 9.5, was used for kinetic measurements. Optically active viologens, **1**−**7**, were prepared by reacting 4,4′-bipyridine with ((*S*)- or ((*R*)-1-phenyl-, (1-(1-naphthyl)-, or (1-cyclo-

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† Dedicated to Professor Ralph G. Wilkins on the occasion of his 70th birthday.

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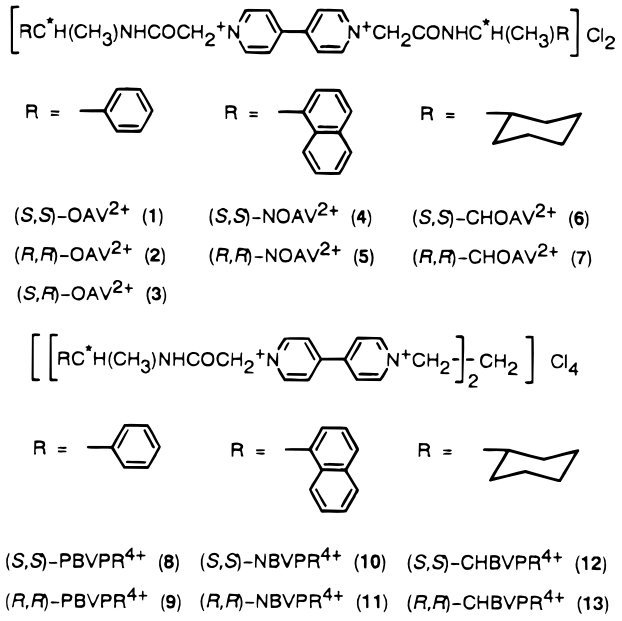
Chart 1



hexylcarbamoyl)bromomethane (**14**) in excess in *N*,*N*-dimethylformamide (DMF), the latter of which was obtained by treating the corresponding chiral-substituted ethylamine with bromoacetic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide in dichloromethane. The (*S*,*R*)-isomer of OAV^{2+} (**3**) was prepared by the reaction of **14** ((*R*)-isomer) with 4,4′-bipyridine in excess, followed by reacting with the (*S*)-isomer of **14** in excess in DMF. The crude products were recrystallized from methanol. Optically active bisviologens, **8**−**13**, were synthesized by the reaction of 1,1′′-trimethylenebis(4,4′-bipyridinium) dibromide[17,18] with the compound **14** in excess in DMF. The crude products were recrystallized from warm water. The details of the synthetic method will be published elsewhere. All of the bromide salts were converted to chloride salts by an anion-exchange chromatography for photochemical measurements. Sodium poly-L-glutamate (poly-L-Glu; mean fw 13 000, Sigma) and sodium poly-D-glutamate (poly-D-Glu; mean fw 15 000, Sigma) were used without further purification. All other chemicals used were of guaranteed grade. 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 ZnMb ($(0.5-2.0) \times 10^{-6}$ M) and quenchers ($0-5.0 \times 10^{-5}$ M) at 25 °C, pH 7.0−8.0 (a 0.01 M sodium phosphate buffer), and $I = 0.02$ and 0.32 M using a Photal RA-412 pulse flash apparatus with a 30 μs pulse-width Xe lamp (λ > 450 nm; a Toshiba Y-47 glass filter). Absorption spectral changes during the reaction were monitored at 460 and 680 nm.

Other Measurements. Cyclic voltammetry was done in a N_2-saturated KCl solution (0.05 M) with a Yanako Model P-900 instrument. A three-electrode system (BAS Inc.) was used with a Pt auxiliary electrode and a glassy carbon or a Pt working electrode against an Ag/AgCl (3.33 M KCl) reference electrode. Electronic absorption spectra were recorded on a Shimadzu UV-240 spectrophotometer. Disproportionation constants of the monoradical trications were determined spectrophotometrically by the same method described in the literature.[18] The pHs of the solutions were measured on a Hitachi-Horiba F-14RS pH meter.

Results and Discussion

The data for electronic absorption, optical rotatory dispersion, and redox potential of the optically active viologens are summarized in Table 1. One of the bisviologens, PTQ^{4+}, having a trimethylene bridge, shows a higher one-electron redox potential (−0.33 V)[18] compared to that of methylviologen (MV^{2+}, −0.45 V).[10,11] This is probably due to the charge effect on viologens. All of the optically active viologen (**1**−**13**) have higher one-electron redox potentials than those of PTQ^{4+} and MV^{2+}, arising from the electron-withdrawing nature of carbamoyl groups of the optically active viologens. The trend in disproportionation of the monoradical cation is as follows: $PTQ^{\bullet 3+}$ ($K_{disp} = 260 \pm 60$)[18] > $CHBVPR^{\bullet 3+}$ (9.9 ± 3.4) > $PBVPR^{\bullet 3+}$ (4.6 ± 1.3) > $NBVPR^{\bullet 3+}$ (2.5 ± 0.4). It has been suggested that the doubly reduced species $PTQ^{2\bullet 2+}$ adopts an intramolecular associated form ("closed form").[18] Disproportionation occurs most readily in $PTQ^{\bullet 3+}$, suggesting greater stability for the closed form of $PTQ^{2\bullet 2+}$. Smaller disproportionation constants for the optically active bisviologens may arise from the steric repulsion between bulk substituents in the intramolecular association than the methyl groups in $PTQ^{2\bullet 2+}$.

Photoinduced ET Reaction of ^{3}(ZnMb)*. Although the excited singlet state of ZnMb was not quenched by all of the viologen used, the ^{3}(ZnMb)* was efficiently quenched with a first-order decay (Figure 1a). Plots of the first-order rate constant of the quenching of ^{3}(ZnMb)*, k_{obsd}, vs the concentrations of viologens were linear (Figures 2 and 3), indicating no appreciable complex formation between ^{3}(ZnMb)* and viologen. When the reaction was monitored at 680 nm, we observed the formation and decay of the radical cation of $ZnMb^{\bullet +}$ (Figure 1b), indicating the ET quenching followed by a thermal back ET reaction, as has been previously observed.[5,19] The decay of $ZnMb^{\bullet +}$ was of second order, indicating that an equimolar amount of $ZnMb^{\bullet +}$ with a viologen radical cation formed. Therefore, the photoinduced ET reaction of ZnMb with viologen is represented in Scheme 1. In the case of bisviologens, the disproportionation of the monoradical cation of bisviologen ($Q^{2+}-Q^{\bullet +}$) is much faster than the back ET reaction.[18] Therefore, the back ET reaction must occur between $ZnMb^{\bullet +}$ and the doubly reduced species of bisviologen ($Q^{\bullet +}-Q^{\bullet +}$), as is shown in Scheme 2.

The quenching rate constant (k_q) was obtained from the slope of the plots of k_{obsd} vs the concentrations of viologens and are listed in Table 2. The thermal back ET reaction was much slower than the quenching reaction of ^{3}(ZnMb)*, and the second-order rate constant of the back ET reaction (k_b) was evaluated at the latter portion of the decay of $ZnMb^{\bullet +}$ at 680 nm after the quenching of ^{3}(ZnMb)* was completed (see Figure 1b), based on the following equation:

$$A_t = (A_0 + k_b[A]_0 A_\infty t)/(1 + k_b[A]_0 t) \qquad (1)$$

Here A_0, A_t, and A_∞ are the absorbances at time 0, *t*, and infinity, respectively, and $[A]_0$ is an initial concentration of $ZnMb^{\bullet +}$. Three unknown parameters, A_0, $k_b[A]_0$, and A_∞, were simultaneously determined. The value of k_b was determined by using the value of $[A]_0$ which was estimated from the concentration of ^{3}(ZnMb)* ($\Delta\epsilon_{428} = \epsilon(\text{ground}) - \epsilon(\text{triplet}) = 1.00 \times 10^5\ M^{-1}\ cm^{-1}$).[5b,20] The values of k_b are listed in Table 3.

Stereoselectivity in Quenching of ^{3}(ZnMb)*. The values of k_q for the (*S*,*S*)-isomers of optically active viologens and bisviologens are larger than those for the (*R*,*R*)-isomers at $I = 0.02$ M. The ratios of $k_q((S,S))/k_q((R,R))$ are 1.5, 1.3, and 1.2 for $NOAV^{2+}$, OAV^{2+}, and $CHOAV^{2+}$, respectively. Therefore, the (*S*,*S*)-isomers preferentially quench ^{3}(ZnMb)*. For the

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Table 1. Electronic Absorption Spectral Data, Specific Rotations, and Redox Potentials of Optically Active Viologens in Water

viologens	λ_{max}/nm (log ϵ)	$[\alpha]_D^{20}$/deg[a]	$E_{12}°$/V[b]	$E_1°$/V	$E_2°$/V	K_{disp}
1	264 (4.33)	−175[c]		−0.20		
2		176[c]				
3		0				
4	260 sh (4.43), 271 (4.49), 278 (4.47), 290 sh (4.28)	−78		−0.17		
5		78				
6	267 (4.34)	−68		−0.20		
7		68				
8	265 (4.83)	−144	−0.21	−0.23[d]	−0.19[d]	4.6 ± 1.3
9		145				
10	260 sh (4.76), 270 (4.78), 280 sh (4.73)	−60	−0.17	−0.18[d]	−0.16[d]	2.5 ± 0.4
11		61				
12	265 (4.70)	−43	−0.21	−0.24[d]	−0.18[d]	9.9 ± 3.4
13		43				
PTQ^{4+} [e]	261 (4.64)	0	−0.26	−0.33[d]	−0.19[d]	260 ± 60

[a] Bromide salts; $c = 0.050$. [b] For two-electron redox process. [c] $c = 0.025$. [d] Determined from the disproportionation constant of monoradical cations. [e] Reference 18.

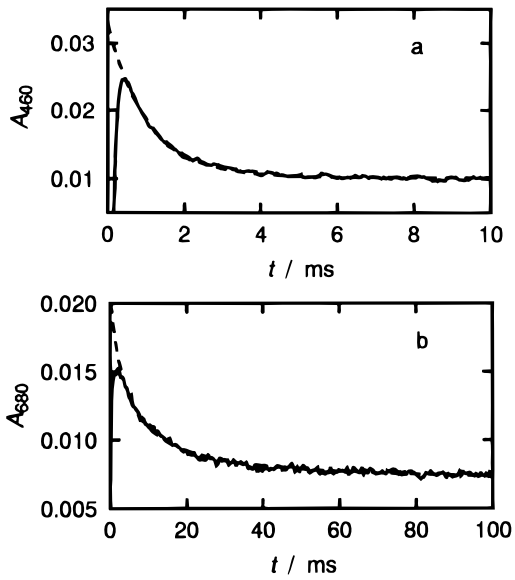

Figure 1. Absorbance changes after irradiation of ZnMb with a Xe flash lamp in the presence of (*R*,*R*)-$NBVPR^{4+}$ (1.5×10^{-5} M) at 25 °C, pH 7.0 (0.01 M phosphate buffer), and $I = 0.02$ M. (a) Decay of ^{3}(ZnMb)* at 460 nm. A dotted line is fitted to the first-order decay kinetics. (b) Decay of $ZnMb^{\bullet+}$ at 680 nm. A dotted line is fitted to eq 1.

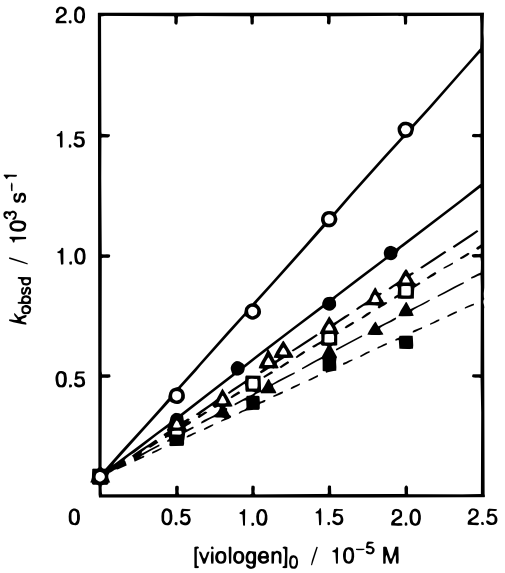

Figure 2. Plots of k_{obsd} vs $[viologen]_0$ for the quenching of ^{3}(ZnMb)* by optically active viologens at 25 °C, pH 7.0 (0.01 M phosphate buffer), and $I = 0.02$ M: (○) (*S*,*S*)-$NOAV^{2+}$; (●) (*R*,*R*)-$NOAV^{2+}$; (□) (*S*,*S*)-OAV^{2+}; (■) (*R*,*R*)-OAV^{2+}; (△) (*S*,*S*)-$CHOAV^{2+}$; (▲) (*R*,*R*)-$CHOAV^{2+}$.

corresponding (*S*,*R*)-isomer of OAV^{2+}, the k_q value is between those for the (*S*,*S*)- and (*R*,*R*)-isomers ($(3.4 \pm 0.2) \times 10^7$ M^{-1} s^{-1} at 25 °C and $I = 0.02$ M). In the case of bisviologens, the stereoselectivity in the (*S*,*S*)-isomers is held, although the ratios of $k_q((S,S))/k_q((R,R))$ become smaller than those for monoviologen: 1.2 for $NBVPR^{4+}$, 1.1 for $PBVPR^{4+}$, and 1.1 for $CHBVPR^{4+}$. This arises from the fact that bisviologen has only one optically active substituent on a viologen unit and that another viologen moiety has achiral methylene groups.

The quenching rate increased with increasing ionic strength, indicating that the reactive site of ^{3}(ZnMb)* is a positively charged site. The ratios of $k_q((S,S))/k_q((R,R))$ became smaller at $I = 0.32$ M, where no stereoselectivity was observed in the OAV^{2+} system. This may arise partially from that the electrostatic repulsion between ^{3}(ZnMb)* and cationic viologen decreases at higher ionic strengths by insertion of chloride ions into the two; thereafter the distance between reactants becomes longer. Therefore, the discrimination of the environment around the optically active viologen will be unclear.

When the pH of the solution increased to 8 at $I = 0.02$ M, the ratios of $k_q((S,S))/k_q((R,R))$ increased in the $NBVPR^{4+}$ system. Increasing pH of the solution causes the deprotonation of ZnMb. Cowan and Gray[22] have reported that the isoelectric point of ZnMb is 7.4 ± 0.2. Therefore, the attractive electrostatic interaction between $NBVPR^{4+}$ and ^{3}(ZnMb)* at pH 8 becomes stronger than that at pH 7.

The quenching of ^{3}(ZnMb)* has been discussed in terms of two mechanisms: (i) The reactant loosely associates or only collides with the protein.[4b] (ii) The reactant diffuses through the protein and the diffusion is gated.[4a] The stereoselectivity found in this work can be explained by both mechanisms. However, we have previously suggested that a cationic quencher attacks the positively charged Lys and/or Arg residue(s) near the heme pocket, thereby inducing a conformational change of ^{3}(ZnMb)* to an active form of the protein, based on lack of the driving force dependence of rates.[5] Large quenchers of viologens and bisviologens may not diffuse into the heme pocket, and the reaction might occur at the surface of the protein. This has been recently confirmed by a time-resolved FT-EPR study.[23] The polypeptide chain of myoglobin has an *S*-configuration and the (*S*,*S*)-isomer of optically active viologen might be more fitted

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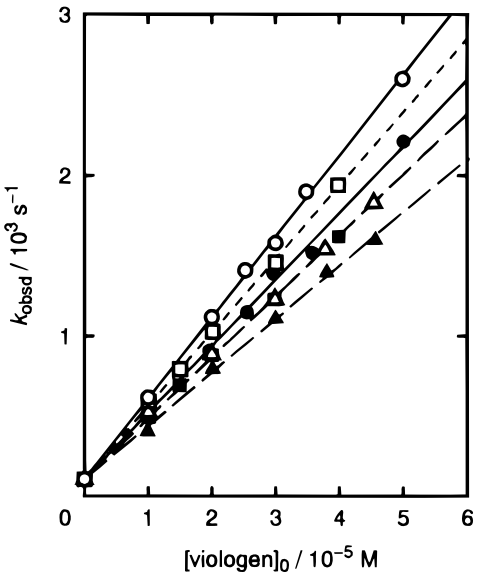

Figure 3. Plots of k_{obsd} vs [viologen]$_0$ for the quenching of ^{3}(ZnMb)* by optically active bis(viologens) at 25 °C, pH 7.0 (0.01 M phosphate buffer), and $I = 0.02$ M: (○) (*S*,*S*)-NBVPR^{4+}; (●) (*R*,*R*)-NBVPR^{4+}; (□) (*S*,*S*)-PBVPR^{4+}; (■) (*R*,*R*)-PBVPR^{4+}; (△) (*S*,*S*)-CHBVPR^{4+}; (▲) (*R*,*R*)-CHBVPR^{4+}.

Scheme 1

$$\text{ZnMb} \underset{k_0}{\overset{h\nu}{\rightleftharpoons}} {}^3(\text{ZnMb})^*$$

$$^3(\text{ZnMb})^* + \text{Q}^{2+} \xrightarrow{k_q} \text{ZnMb}^{+\cdot} + \text{Q}^{+\cdot}$$

$$\text{ZnMb}^{+\cdot} + \text{Q}^{+\cdot} \xrightarrow{k_b} \text{ZnMb} + \text{Q}^{2+}$$

Scheme 2

$$\text{ZnMb} \underset{k_0}{\overset{h\nu}{\rightleftharpoons}} {}^3(\text{ZnMb})^*$$

$$^3(\text{ZnMb})^* + \text{Q}^{2+}\text{–Q}^{2+} \xrightarrow{k_q} \text{ZnMb}^{+\cdot} + \text{Q}^{2+}\text{–Q}^{+\cdot}$$

$$2\,\text{Q}^{2+}\text{–Q}^{+\cdot} \overset{K_{disp}}{\rightleftharpoons} \text{Q}^{2+}\text{–Q}^{2+} + \text{Q}^{+\cdot}\text{–Q}^{+\cdot}$$

$$\text{ZnMb}^{+\cdot} + \text{Q}^{+\cdot}\text{–Q}^{+\cdot} \xrightarrow{k_b} \text{ZnMb} + \text{Q}^{2+}\text{–Q}^{+\cdot}$$

to the reactive site of myoglobin than the (*R*,*R*)-isomer. The interaction of (*S*,*S*)-OAV^{2+} with ^{3}(ZnMb)* is expected to be stronger than that of (*R*,*R*)-OAV^{2+}; therefore, the conformational change of ^{3}(ZnMb)* may be induced by (*S*,*S*)-OAV^{2+} more effectively. The naphthyl group of NOAV^{2+} is more bulky than the phenyl group of OAV^{2+}. NOAV^{2+} quenches ^{3}(ZnMb)* faster than OAV^{2+}, although the redox potentials of NOAV^{2+} and OAV^{2+} are similar to another: −0.18 V for the former and −0.20 V for the latter. Therefore, the steric bulk of the substituents of viologen may induce the conformational change of ^{3}(ZnMb)* more effectively due to the steric repulsion between naphthyl groups and the polypeptide chain of ^{3}(ZnMb)*. We suggest that steric acceleration takes place in these systems. Since the steric bulk of cyclohexyl groups is not so large as that of naphthyl groups, the quenching rate is smaller than that for the NOAV^{2+} systems and similar to that for OAV^{2+}. The stereoselectivity in CHOAV^{2+} is also less effective than that for NOAV^{2+}.

Effect of Poly-L-Glutamate on Quenching of ^{3}(ZnMb)*. Poly-L-glutamate ion is a highly charged anion and randomly coiled at a neutral pH region in aqueous solutions.[21] We have examined the effect of optically active poly-L-glutamate ions on the quenching of ^{3}(ZnMb)* by OAV^{2+} and NOAV^{2+} (Figure 4). The quenching rate decreased with an increase in the concentrations of sodium poly-L-glutamate and became constant at [poly-L-Glu]$_0 \geq 9 \times 10^{-6}$ M, where no stereoselectivity was observed for both OAV^{2+} and NOAV^{2+} systems. The same behavior was observed in the quenching of ^{3}(ZnMb)* by (*R*,*R*)-NOAV^{2+} in the presence of poly-D-glutamate ions (Figure 4). The estimated rate constants of the quenching of ^{3}(ZnMb)* by viologen bound to poly-Glu are as follows, 2.8×10^7 M^{-1} s^{-1} for the (*S*,*S*)- and (*R*,*R*)-OAV^{2+}/poly-L-Glu systems and 3.3×10^7 M^{-1} s^{-1} for the (*S*,*S*)- and (*R*,*R*)-NOAV^{2+}/poly-L-Glu and poly-D-Glu systems, respectively. We also found from the

Table 2. Rate Constants for the Quenching Reactions of ^{3}(ZnMb)* by Optically Active Viologens at 25 °C, pH 7.0 (a 0.01 M Phosphate Buffer), and $I = 0.02$ M

	$k_q/10^7$ M^{-1} s^{-1}		
viologen	(*S*,*S*)-isomer	(*R*,*R*)-isomer	$k_q(S,S)/k_q(R,R)$
OAV^{2+}	3.9 ± 0.1	2.9 ± 0.1	1.3
OAV^{2+} [a]	4.9 ± 0.1	4.9 ± 0.1	1.0
OAV^{2+} [b]	4.2 ± 0.1	3.3 ± 0.1	1.3
OAV^{2+} [c]	2.8 ± 0.1	2.8 ± 0.1	1.0
OAV^{2+} [d]	2.8 ± 0.1	2.8 ± 0.1	1.0
NOAV^{2+}	7.1 ± 0.2	4.8 ± 0.2	1.5
NOAV^{2+} [a]	9.3 ± 0.3	6.6 ± 0.3	1.4
NOAV^{2+} [e]	7.6 ± 0.3	5.4 ± 0.2	1.4
NOAV^{2+} [f]	3.3 ± 0.1	3.3 ± 0.1	1.0
NOAV^{2+} [g]	3.3 ± 0.1	3.3 ± 0.1	1.0
CHOAV^{2+}	4.2 ± 0.1	3.4 ± 0.1	1.2
MV^{2+} [h]	4.2 ± 0.1		
PBVPR^{4+}	4.6 ± 0.3	3.8 ± 0.3	1.1
NBVPR^{4+}	5.1 ± 0.5	4.2 ± 0.4	1.2
NBVPR^{4+} [a]	10.0 ± 0.6	9.0 ± 0.5	1.1
NBVPR^{4+} [i]	6.4 ± 0.6	5.1 ± 0.5	1.3
CHBVPR^{4+}	3.8 ± 0.3	3.3 ± 0.2	1.1
PTQ^{4+} [h]	0.32 ± 0.03		

[a] At $I = 0.32$ M. [b] At $I = 0.05$ M. [c] In the presence of 9.0×10^{-6} M poly-L-Glu at $I = 0.05$ M. [d] In the presence of 9.0×10^{-6} M poly-D-Glu at $I = 0.05$ M. [e] At $I = 0.09$ M. [f] In the presence of 9.0×10^{-6} M poly-L-Glu at $I = 0.09$ M. [g] In the presence of 9.0×10^{-6} M poly-D-Glu at $I = 0.09$ M. [h] Reference 5. [i] At pH 8.0.

Table 3. Rate Constants for the Back ET reactions between ZnMb$^{+\cdot}$ and Optically Active Viologen-Radical Cations at 25 °C, pH 7.0 (a 0.01 M Phosphate Buffer), and $I = 0.02$ M

	$k_b/10^8$ M^{-1} s^{-1}		
viologen	(*S*,*S*)-isomer	(*R*,*R*)-isomer	$k_b(S,S)/k_b(R,R)$
OAV^{2+}	1.4 ± 0.1	0.98 ± 0.08	1.4
OAV^{2+} [a]	1.8 ± 0.1	1.3 ± 0.1	1.4
OAV^{2+} [b]	1.5 ± 0.1	1.1 ± 0.1	1.4
OAV^{2+} [c]	0.65 ± 0.05	0.65 ± 0.05	1.0
OAV^{2+} [d]	0.65 ± 0.05	0.65 ± 0.05	1.0
NOAV^{2+}	2.5 ± 0.1	1.7 ± 0.1	1.4
NOAV^{2+} [a]	2.6 ± 0.2	1.9 ± 0.1	1.4
NOAV^{2+} [e]	2.5 ± 0.3	1.8 ± 0.1	1.4
NOAV^{2+} [f]	1.1 ± 0.1	1.1 ± 0.1	1.0
NOAV^{2+} [g]	1.1 ± 0.1	1.1 ± 0.1	1.0
CHOAV^{2+}	1.8 ± 0.2	1.4 ± 0.1	1.3
MV^{2+} [h]	0.40 ± 0.04		
PBVPR^{4+}	1.3 ± 0.1	0.95 ± 0.06	1.3
NBVPR^{4+}	1.6 ± 0.1	1.4 ± 0.1	1.2
NBVPR^{4+} [a]	2.6 ± 0.1	2.3 ± 0.1	1.1
NBVPR^{4+} [i]	1.8 ± 0.1	1.3 ± 0.1	1.4
CHBVPR^{4+}	1.2 ± 0.1	0.93 ± 0.06	1.3
PTQ^{4+} [h]	0.24 ± 0.02		

[a] At $I = 0.32$ M. [b] At $I = 0.05$ M. [c] In the presence of 9.0×10^{-6} M poly-L-Glu at $I = 0.05$ M. [d] In the presence of 9.0×10^{-6} M poly-D-Glu at $I = 0.05$ M. [e] At $I = 0.09$ M. [f] In the presence of 9.0×10^{-6} M poly-L-Glu at $I = 0.09$ M. [g] In the presence of 9.0×10^{-6} M poly-D-Glu at $I = 0.09$ M. [h] Reference 5. [i] At pH 8.0.

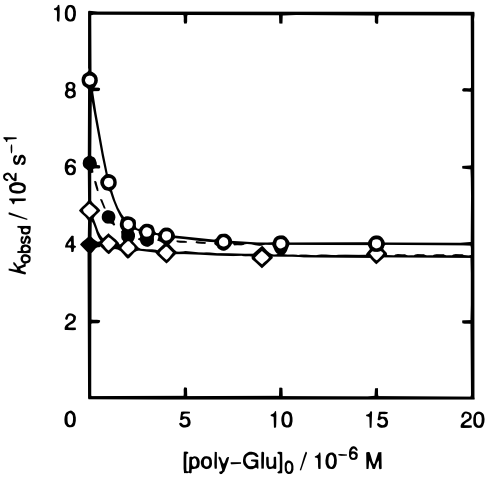

Figure 4. Effect of polyglutamate ions for the quenching of ^{3}(ZnMb)* by optically active viologens (1.0×10^{-5} M) at 25 °C and pH 7.0 (0.01 M phosphate buffer): (○) (*S*,*S*)-NOAV^{2+}/poly-L-Glu at $I = 0.09$ M; (●) (*R*,*R*)-NOAV^{2+}/poly-D-Glu at $I = 0.09$ M; (◇) (*S*,*S*)-OAV^{2+}/poly-L-Glu at $I = 0.05$ M; (◆) (*R*,*R*)-OAV^{2+}/poly-L-Glu at $I = 0.05$ M.

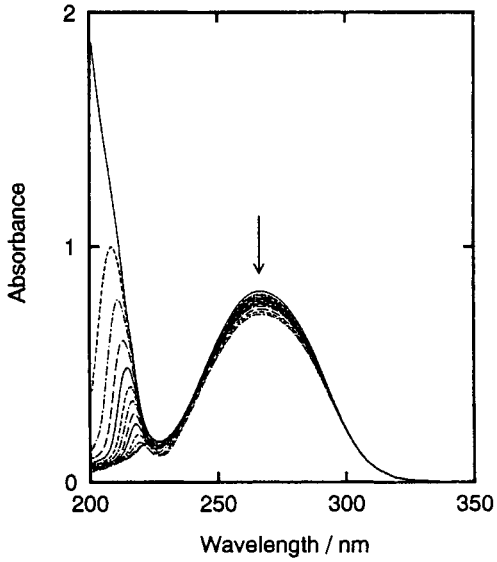

Figure 5. Absorption spectral change of (*S*,*S*)-OAV^{2+} (3.8×10^{-5} M) by adding poly-L-Glu at 25 °C, pH 7.0 (0.01 M phosphate buffer), and $I = 0.05$ M. The spectra were measured against the solutions containing the same concentrations of poly-L-Glu. Concentrations (M): (1) 0; (2) 5.7×10^{-6}; (3) 1.13×10^{-5}; (4) 1.68×10^{-5}; (5) 2.24×10^{-5}; (6) 2.79×10^{-5}; (7) 3.33×10^{-5}; (8) 3.88×10^{-5}; (9) 4.42×10^{-5}; (10) 5.48×10^{-5}; (11) 6.54×10^{-5}; (12) 8.61×10^{-5}; (13) 1.06×10^{-4}; (14) 1.26×10^{-4}.

spectrophotometric measurements that (*S*,*S*)-OAV^{2+} binds poly-L-Glu more strongly than (*R*,*R*)-OAV^{2+}; there is stereoselectivity in the association between OAV^{2+} and poly-L-Glu, the latter having an *S*-configuration. Adding poly-L-Glu into the solution of OAV^{2+} resulted in the spectral change of OAV^{2+}, arising from the ion-pair formation (Figure 5). The binding constants for the 1:1 complex of OAV^{2+} with poly-L-Glu are determined from eq 2 under the condition that [poly-L-Glu]$_0$ >> [OAV^{2+}]$_0$.

$$1/|A - A_0| = 1/|A_\infty - A_0| + 1/(|A_\infty - A_0|K[\text{poly-L-Glu}]_0) \quad (2)$$

Here A_0, A_∞, and A are the absorbances of OAV^{2+}, the complex

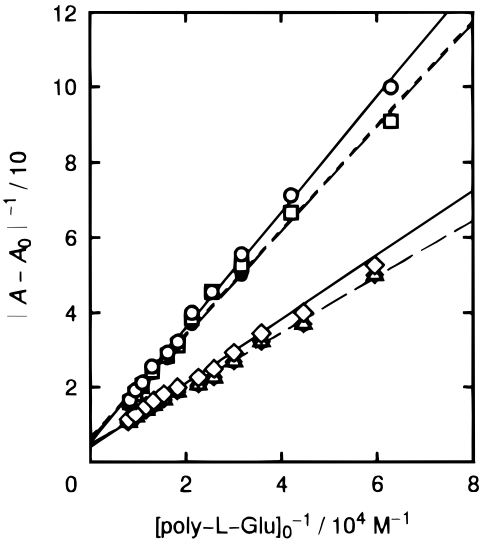

Figure 6. Plots of $|A - A_\infty|^{-1}$ vs [poly-L-Glu]$_0^{-1}$ for the OAV^{2+}/poly-L-Glu systems: (○) (*R*,*R*)-OAV^{2+} at 270 nm; (□) (*R*,*R*)-OAV^{2+} at 264 nm; (●) (*R*,*R*)-OAV^{2+} at 260 nm; (◇) (*S*,*S*)-OAV^{2+} at 270 nm; (◆) (*S*,*S*)-OAV^{2+} at 264 nm; (△) (*S*,*S*)-OAV^{2+} at 260 nm.

of OAV^{2+} with poly-L-Glu, and the equilibrium mixture of the free and bound OAV^{2+} at the given wavelengths (260, 264, and 270 nm). The plots of $1/|A - A_0|$ vs 1/[poly-L-Glu]$_0$ are shown in Figure 6. The value of K was obtained from the intercept/slope: $K = (5.9 \pm 0.6) \times 10^3$ M^{-1} ((*S*,*S*)-OAV^{2+}), $(4.3 \pm 0.5) \times 10^3$ M^{-1} ((*R*,*R*)-OAV^{2+}), $(5.7 \pm 0.5) \times 10^3$ M^{-1} ((*S*,*S*)-NOAV^{2+}), and $(4.2 \pm 0.4) \times 10^3$ M^{-1} ((*R*,*R*)-NOAV^{2+}) at 25 °C and $I = 0.05$ M (NaCl), respectively. The formation of the complex between OAV^{2+} or NOAV^{2+} and poly-L-Glu decreases the quenching rate of ^{3}(ZnMb)* due to an increase in the separation distance of the redox partners. Therefore, the rate constant of the quenching of ^{3}(ZnMb)* by (*S*,*S*)-isomers of the optically active viologens bound to poly-L-Glu is smaller than that for the free viologens.

Stereoselectivity in the Back ET Reaction. Back ET reactions between ZnMb$^{\bullet+}$ and viologen radical cations have also stereoselectivity for the (*S*,*S*)-isomers. The ratios of k_q((*S*,*S*))/k_q((*R*,*R*)) are 1.1−1.4, being insensitive to ionic strength, although the stereoselectivity for the (*S*,*S*)-isomers becomes higher at pH 8. In the presence of poly-L-Glu, the back ET reaction becomes slower and the stereoselectivity lower, suggesting that the separation distance between reactants becomes longer due to the interaction of poly-L-Glu and viologen radical cations. The effect of charge on viologen radical cations is less important in the back ET reaction, because the reactive species of bisviologen seems to be the doubly reduced species (Q$^{\bullet+}$−Q$^{\bullet+}$). Steric bulk of the substituents of viologen may be as important as the quenching process.[5]

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Supporting Information Available: Tables SI−SIV (kinetic data) (7 pages). Ordering information is given on any current masthead page.

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