

Stereoselective Electron-Transfer Reactions of Myoglobin and Cytochrome *c* with Chiral Viologen-Radical Cations

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Electron-transfer (ET) reactions of metmyoglobin (metMb) and the oxidized form of cytochrome *c* (cyt *c*(III)) with chiral viologen-radical cations containing (1-phenyl- and 1-cyclohexylethyl)carbamoylmethyl groups were examined at pH 7.0 (10 mM phosphate buffer), ionic strengths (*I*) of 0.040–0.50 M, and temperatures of 10–35 °C. In the metMb system, the pseudo first-order rate constant of the decay of the viologen-radical cation was saturated with increasing concentrations of metMb, indicating that metMb interacts with the viologen-radical cation to form a complex followed by an intramolecular ET reaction. The stereoselectivity was observed in both the complex formation and the intramolecular ET processes for the (*S*,*S*)-isomers of chiral viologens. The ratios of the association constants and the intramolecular ET rate constants of the (*S*,*S*)-isomer with those of the (*R*,*R*)-isomer were 1.1–1.3 and 1.1–1.5, respectively. The saturation kinetics was not observed for the ET reaction between metMb and the achiral benzylviologen-radical cation, which has no CONH bond, suggesting that the hydrogen bonding of the CONH group of the chiral viologen with the side chain of the polypeptide in metMb plays an important role in the stereoselectivity. No appreciable complex formation was observed in the ET reaction between cyt *c*(III) and viologen-radical cations, although the stereoselectivity was observed. The ratios of the second-order rate constants of the (*S*,*S*)-isomer with those of the (*R*,*R*)-isomer were 1.1–1.4. The stereoselectivity is discussed based on the activation parameters.

The electron-transfer (ET) reactions of metalloproteins have received considerable attention in both fields of chemistry and biology.[1–6] Specific recognition and binding between a protein and a small molecule or ion occur in a biological bimolecular ET reaction, because chirality is an obvious property of reaction sites in proteins. Since the first demonstration of stereoselectivity in an outer-sphere ET reaction of metal complex,[7] asymmetry induction has been established with a number of examples of metal complexes. Furthermore, stereoselectivity in the ET reactions between metalloproteins and chiral metal complexes has been reported.[8–13] There are, however, very few reports on the stereoselectivity in the ET reactions of metalloproteins with chiral organic redox reagents. One of the reasons is a lack of systematic synthesis of such chiral materials.[14]

Viologens are diquaternary salts of 4,4′-bipyridine, and have been used extensively as mediators in the catalytic photolysis of water under visible light with a sensitizer.[15] We have recently reported a systematic synthesis of chiral viologens containing 1-(1-naphthyl)-, 1-phenyl-, and 1-cyclohexylethylamines[16] and found stereoselectivity in the ET quenching of the excited triplet state of zinc-substituted myoglobin (^{3}(ZnMb)*) by the chiral viologens, where there is no evidence for complex formation between ^{3}(ZnMb)* and viologens.[17,18] This may arise partly because charge repulsion prevents a strong interaction between the two. Viologen is easily reduced by one-electron reducing agents, chemically or photochemically, to form a stable radical cation, whose formal charge decreases. Therefore, the use of the viologen-radical cation should improve monitoring such interactions. Myoglobin is a weak basic hemoprotein, whose physiological function is oxygen storage and its redox potential is 0.06 V vs NHE.[19] In this work we report that metmyoglobin (metMb) interacts with chiral viologen-radical cations ($OAV^{\bullet+}$ and $CHOAV^{\bullet+}$, Chart 1) to form a complex. Therefore, we can investigate the stereoselectivity in both the precursor complex formation and the intrinsic ET processes. The redox potentials for $OAV^{2+}/OAV^{\bullet+}$ and $CHOAV^{2+}/CHOAV^{\bullet+}$ are the same ($E^0 = -0.20$ V).[16–18]

Cytochrome *c* (cyt *c*) is a strong basic and ET protein whose formal charge is +7 for the oxidized form (cyt *c*(III)) and its redox potential is 0.26 V.[20] We also investigated the ET reaction of cyt *c*(III) with the chiral viologen radical-cation to check the effect of the charge of the protein. The chiral viologens used in this work contained CONH groups. To investigate the role of the CONH groups we also examined the ET re-

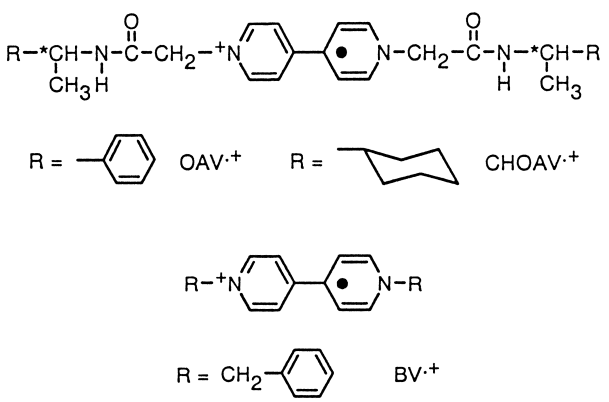


Chart 1.

action of these hemoproteins with an achiral benzylviologen-radical cation ($E^0 = -0.36$ V for $BV^{2+}/BV^{\bullet+}$).[21] A preliminary communication has been published elsewhere.[22]

Experimental

Materials. Horse heart metMb (Sigma) and cyt *c*(III) (Wako Pure Chemical Industries, Ltd.) were purified as previously described.[22–24] Bromide salts of the chiral viologens were synthesized by a previously reported method, and were converted to chloride salts by anion-exchange chromatography.[16] The radical cations, $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$, were prepared in situ by photochemical reduction of the parent viologens[16] with the excited triplet state of tris(2,2′-bipyridine)ruthenium(II) ($^3([Ru(bpy)_3]^{2+})^*$) in the presence of a disodium salt of ethylenediaminetetraacetic acid (Na_2H_2edta) by freeze-pump-thaw cycles to remove a trace amount of dioxygen. Benzylviologen dichloride (1,1′-dibenzyl-4,4′-bipyridinium dichloride, $[BV]Cl_2$) was purchased from Aldrich Chemical Co., Inc. 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. A single flash photolysis was performed in deaerated solutions containing metMb or cyt *c*(III) ((0.30–3.00) $\times 10^{-5}$ M, 1 M = 1 mol dm^{-3}), the parent viologen ((0.50–2.00) $\times 10^{-3}$ M), $[Ru(bpy)_3]^{2+}$ (1.00×10^{-5} M), and Na_2H_2edta (5.00×10^{-3} M) at 10.0–35.0 °C, pH 7.0 (20 mM phosphate buffer), and I = 0.040–0.50 M 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). The absorption spectral changes during the reaction were monitored at 602 nm (the decay of viologen-radical cation), 409 nm (the decay of metMb), 434 nm (the formation of deoxyMb), 530 nm (the decay of cyt *c*(III)), and 549 nm (the formation of cyt *c*(II)). Under the present experimental conditions, $^3([Ru(bpy)_3]^{2+})^*$ reacted only with viologen to produce the (2.0–5.0) $\times 10^{-7}$ M viologen-radical cation.

Other Measurements. The electronic absorption spectra were recorded on Shimadzu UV-240 and MultiSpec-1500 spectrophotometers. The pHs of the solutions were measured on a Hitachi-Horiba F-14RS pH meter.

Results and Discussion

Cytochrome *c*. Figure 1 shows the absorption spectral changes after irradiation by light of a degassed solution containing cyt *c*(III), (*R*,*R*)-OAV^{2+}, $[Ru(bpy)_3]^{2+}$, and Na_2H_2edta at 25.0 °C, pH 7.0 (20 mM phosphate buffer), and I = 0.040 M (NaCl). The same first-order rate constant was obtained from traces of the absorbance change vs. time for the formation of cyt *c*(II) and the decays of cyt *c*(III) and (*R*,*R*)-$OAV^{\bullet+}$. The observed first-order rate constant (k_{obsd}) was linearly dependent on the initial concentrations of cyt *c*(III) ([cyt *c*(III)]$_0$), as shown in Fig. 2, indicating no appreciable complex formation

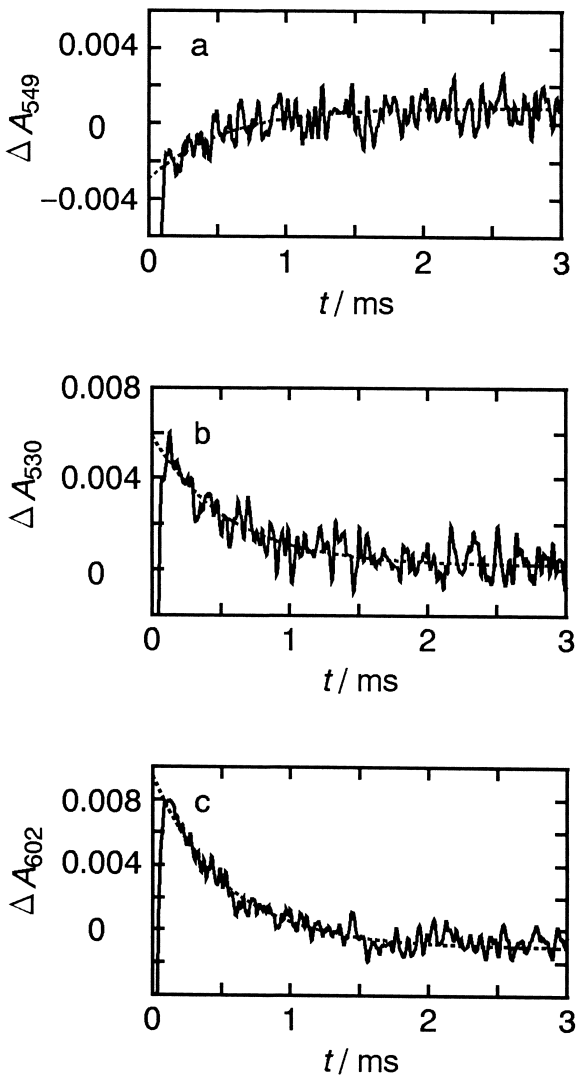


Fig. 1. Absorption spectral changes for the reaction of cyt *c*(III) (3.00×10^{-5} M) with (*R*,*R*)-$OAV^{\bullet+}$ at 25 °C and I = 0.040 M. (a) Formation of cyt *c*(II) at 549 nm. (b) Decay of cyt *c*(III) at 530 nm. (c) Decay of (*R*,*R*)-$OAV^{\bullet+}$ at 602 nm. Dotted lines are fitted to the first-order kinetics.

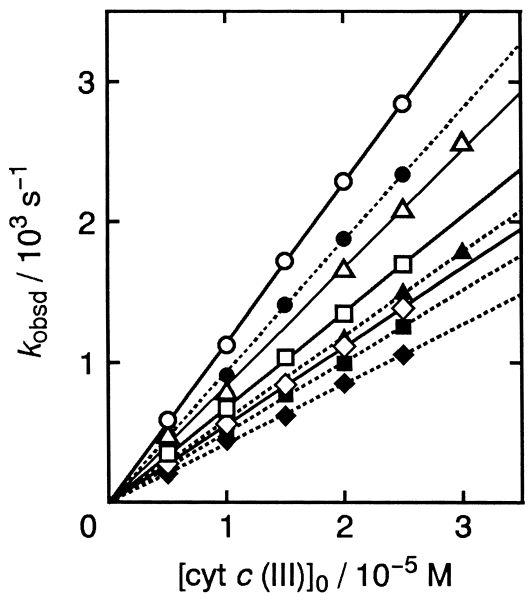


Fig. 2. Plots of k_{obsd} vs [cyt *c*(III)]$_0$ for the reaction of cyt *c*(III) with $OAV^{\bullet+}$ at pH 7.0 and I = 0.040 M. (◇, ◆) 10 °C, (□, ■) 15 °C, (△, ▲) 25 °C, and (○, ●) 35 °C. Open and closed symbols are for (*S*,*S*)- and (*R*,*R*)-isomers, respectively.

Table 1. Rate Constants of the Reactions of cyt *c*(III) with $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ at pH 7.0 (0.020 M Phosphate Buffer)

			$k/10^8$ M^{-1} s^{-1}		
Viologen	Temp/°C	*I*/M	(*S*,*S*)-isomer	(*R*,*R*)-isomer	*k*(*S*,*S*)/*k*(*R*,*R*)
$OAV^{\bullet+}$	10.0	0.040	0.56 ± 0.03	0.43 ± 0.03	1.3 ± 0.1
	15.0	0.040	0.68 ± 0.05	0.51 ± 0.03	1.4 ± 0.1
	25.0	0.040	0.84 ± 0.06	0.60 ± 0.04	1.4 ± 0.1
		0.10	1.1 ± 0.1	0.82 ± 0.06	1.3 ± 0.1
		0.30	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
		0.40	1.8 ± 0.1	1.7 ± 0.1	1.1 ± 0.1
	35.0	0.040	1.1 ± 0.1	0.94 ± 0.06	1.2 ± 0.1
$CHOAV^{\bullet+}$	10.0	0.040	0.80 ± 0.06	0.56 ± 0.04	1.4 ± 0.1
	15.0	0.040	0.88 ± 0.07	0.63 ± 0.05	1.4 ± 0.1
	25.0	0.040	0.98 ± 0.07	0.74 ± 0.05	1.3 ± 0.1
		0.10	1.2 ± 0.1	0.83 ± 0.06	1.4 ± 0.1
		0.30	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
		0.40	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
	35.0	0.040	1.1 ± 0.1	0.86 ± 0.06	1.3 ± 0.1

between cyt *c*(III) and $OAV^{\bullet+}$,

$$\text{cyt } c(\text{III}) + OAV^{\bullet+} \rightarrow \text{cyt } c(\text{II}) + OAV^{2+} \; k. \quad (1)$$

Therefore, the reaction obeys the following rate law:

$$-\text{d}[OAV^{\bullet+}]/\text{d}t = -\text{d}[\text{cyt } c(\text{III})]/\text{d}t = k[\text{cyt } c(\text{III})][OAV^{\bullet+}]. \quad (2)$$

The second-order rate constant (*k*) was evaluated from the slope of the straight line, and is given in Table 1. The same behavior was observed for the reactions of cyt *c*(III) with $CHOAV^{\bullet+}$. The data are also summarized in Table 1. The second-order rate constants for the (*S*,*S*)-isomers of chiral viologen-radical cations (*k*(*S*,*S*)) are larger than those for the (*R*,*R*)-isomers (*k*(*R*,*R*)) by 1.1–1.4, indicating the presence of the stereoselectivity for the (*S*,*S*)-isomers in the ET reactions of cyt *c*(III) with the chiral viologen-radical cations.

The second-order rate constant increases with increasing ionic strengths, suggesting that the reactive sites of the reactants are positively charged. Since the charge on the viologen-radical cation is +1, the reactive sites on cyt c(III) must be positively charged Lys and/or Arg residues. The stereoselectivity decreased slightly with increasing ionic strengths. The amino acid residue of the polypeptide chain of cyt *c*(III) has an *S*-configuration and the (*S*,*S*)-isomer of chiral viologen-radical cation might be more fitted to the reactive site of cyt *c*(III) than the (*R*,*R*)-isomer. The interaction of (*S*,*S*)-isomer with cyt *c*(III) is expected to be stronger than that of the (*R*,*R*)-isomer; therefore, the overall reaction rate may be accelerated for the former.

Myoglobin. The same first-order rate constant was obtained from the traces of the absorbance change vs. time for the formation of deoxyMb and the decays of metMb and viologen-radical cations.[22] The observed first-order rate constant of k_{obsd} was saturated with increasing the concentrations of metMb at a variety of ionic strengths (Fig. 3). The reaction rate also increased with increasing ionic strengths for this system, indicating that the reactive species are positively charged as well as the cyt *c*(III) system. The pK_a values for the acid-dissociation of the coordinated water for horse heart metMb are

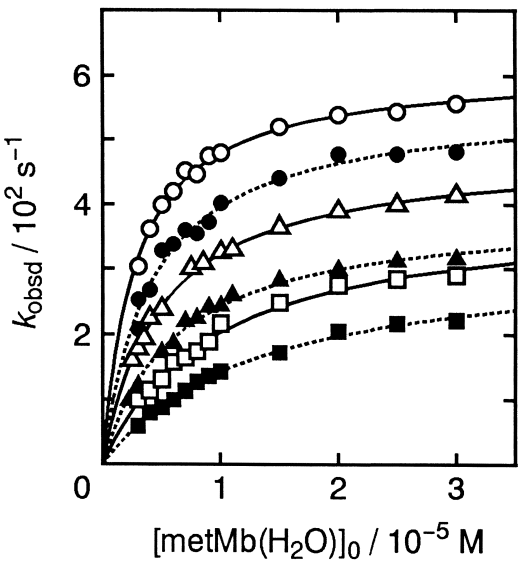


Fig. 3. Plots of k_{obsd} vs $[metMb]_0$ for the reaction of metMb with $OAV^{\bullet+}$ at 25 °C and pH 7.0. (□, ■) I = 0.040 M, (△, ▲) I = 0.10 M, and (○, ●) I = 0.50 M. Open and closed symbols are for (*S*,*S*)- and (*R*,*R*)-isomers, respectively.

8.82–9.03 at 25.0 °C and I = 0.10–1.0 M. Therefore, the predominant species of metMb at pH 7.0 is metMb(H_2O).[24] It is, therefore, demonstrated that metMb interacts with the viologen-radical cation to form a complex followed by an *intra*complex ET reaction, as shown in Scheme 1. From the above mechanism, k_{obsd} is represented by

$$k_{obsd} = (k^{intra}k_1/k_{-1})[metMb]_0/ \{1 + (k^{intra}/k_{-1}) + (k_1/k_{-1})[metMb]_0\}. \quad (3)$$

When $k^{intra} \ll k_{-1}$, the following equation is derived:

$$k_{obsd} = (k^{intra}k_1/k_{-1})[metMb]_0/\{1 + (k_1/k_{-1})[metMb]_0\}. \quad (4)$$

Here, $[metMb]_0$ is the initial concentration of metMb. The

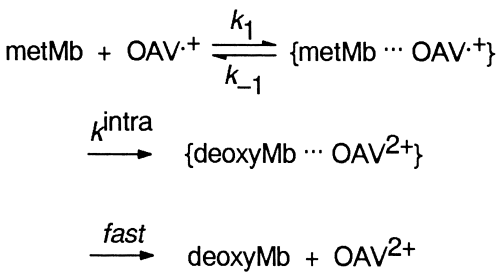
Table 2. Intramolecular ET Rate Constant (k^{intra}) and the Association Constant (K_1) for the Reactions of metMb with $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ at pH 7.0 (0.020 M Phosphate Buffer)

			$k^{intra}/10^2$ s^{-1}			$K_1/10^5$ M^{-1}		
Viologen	Temp/°C	*I*/M	(*S*,*S*)-isomer	(*R*,*R*)-isomer	$k^{intra}(S,S)/k^{intra}(R,R)$	(*S*,*S*)-isomer	(*R*,*R*)-isomer	$K_1(S,S)/K_1(R,R)$
$OAV^{\bullet+}$	10.0	0.040	2.9 ± 0.2	1.9 ± 0.1	1.5	1.4 ± 0.1	1.1 ± 0.1	1.3
	15.0	0.040	3.5 ± 0.2	2.4 ± 0.1	1.5	1.1 ± 0.1	1.0 ± 0.1	1.1
	25.0	0.040	4.0 ± 0.2	3.2 ± 0.2	1.3	1.0 ± 0.1	0.80 ± 0.05	1.3
		0.10	4.8 ± 0.3	3.9 ± 0.3	1.2	2.0 ± 0.1	1.7 ± 0.1	1.2
		0.50	6.1 ± 0.3	6.0 ± 0.3	1.0	3.6 ± 0.2	2.4 ± 0.1	1.5
	35.0	0.040	5.0 ± 0.3	4.4 ± 0.3	1.1	0.82 ± 0.05	0.72 ± 0.05	1.1
$CHOAV^{\bullet+}$	25.0	0.10	3.0 ± 0.2	2.2 ± 0.1	1.4	1.2 ± 0.1	0.94 ± 0.05	1.3
		0.50	4.2 ± 0.2	3.8 ± 0.2	1.1	1.4 ± 0.1	1.2 ± 0.1	1.2

Table 3. Activation Parameters for the Reactions of cyt *c*(III) with $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ at pH 7.0 and $I = 0.040$ M

Viologen	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J mol^{-1} K^{-1}	$\Delta\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta\Delta S^{\ddagger}$/J mol^{-1} K^{-1}	$T\Delta\Delta S^{\ddagger}$/kJ mol^{-1}
$OAV^{\bullet+}$					
(*S*,*S*)-	17.4 ± 1.2	−34.5 ± 2.4			
(*R*,*R*)-	19.2 ± 1.3	−30.6 ± 2.1			
			−1.8	−3.9	−1.2[a)]
$CHOAV^{\bullet+}$					
(*S*,*S*)-	6.5 ± 0.5	−70.0 ± 4.9			
(*R*,*R*)-	10.2 ± 0.7	−60.3 ± 4.2			
			−3.7	−9.7	−2.9[a)]

a) At 25 °C.



Scheme 1.

values of k^{intra} and K_1 ($= k_1/k_{-1}$) are listed in Table 2. Both values of k^{intra} and K_1 for the (*S*,*S*)-isomers are larger than those for the (*R*,*R*)-isomers. The ratios are 1.1–1.3 for k^{intra} and 1.1–1.5 for K_1, indicating that there is stereoselectivity in both the association and intramolecular ET processes.

The increase in K_1 with increasing ionic strengths means that the association becomes stronger with increasing ionic strengths, arising from decreasing the charge repulsion. On the other hand, the ionic-strength dependence of the intramolecular ET rate constant (k^{intra}) in Scheme 1 cannot be simply interpreted. Usually, the ionic-strength dependence of the rate constant has been found in a bimolecular ET reaction.[26] However, a similar behavior was observed for a few intramolecular ET systems.[27–30] The following two interpretations are possible. First, if the present intramolecular ET process contained a rearrangement within a precursor complex of metMb with a viologen-radical cation, any breakup and reformation of the precursor complex prior to ET should depend on the ionic strength.[31] Second, the free-energy change and the outer-sphere reorganization energy of the ET process may depend on the ionic strength.[30] Redox potentials of viologens are not sensitive to the ionic strength and the pH.[32] Therefore, the ionic-strength effect in the present intramolecular ET system might arise from a gating mechanism,[17,18,31,33–42] where the rearrangement of metMb and viologen-radical cations is a rate-determining step.

Temperature Dependence. The temperature dependence of the rate constants for the reactions of cyt *c*(III) with $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ is summarized in Table 1. The activation enthalpy and entropy were obtained from the slope and intercept of the straight lines, respectively, and are summarized in Table 3. In the present system the $\Delta H^{\ddagger}$ values for the (*S*,*S*)-isomers are smaller than those for the (*R*,*R*)-isomers, while the $\Delta S^{\ddagger}$ values are more negative than those for the (*R*,*R*)-isomers. The difference between the $\Delta H^{\ddagger}$ values for the (*S*,*S*)- and (*R*,*R*)-isomers ($\Delta\Delta H^{\ddagger}$) are −1.8 and −3.7 kJ mol^{-1} for the $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ systems, respectively. The values of $\Delta\Delta S^{\ddagger}$ are −3.9 and −9.7 J mol^{-1} K^{-1} for the $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ systems, respectively. At 25 °C, the contribution of $\Delta\Delta H^{\ddagger}$ is larger than that of $\Delta\Delta S^{\ddagger}$. Since the lower $\Delta H^{\ddagger}$ and the more positive $\Delta S^{\ddagger}$ accelerate the reaction, the stereoselectivity for the (*S*,*S*)-isomer is predominantly controlled by $\Delta H^{\ddagger}$.

A substituent effect on the rate of the reduction of cyt *c*(III) by $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ is small. This is reasonable because the redox potentials of $OAV^{2+/\bullet+}$ and $CHOAV^{2+/\bullet+}$ are the same: −0.20 V.[16–18] However, the activation parameters are different; both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for $CHOAV^{\bullet+}$ are lower than those for $OAV^{\bullet+}$. This is probably due to stabilization of the precursor complex by the interaction of an aliphatic residue on the surface of cyt *c*(III), such as Lys, with the cyclohexyl group of $CHOAV^{\bullet+}$.

The temperature dependence on the reactions of metMb

Table 4. Activation Parameters for the Reaction of metMb with $OAV^{\bullet+}$ at pH 7.0 and $I = 0.040$ M

$OAV^{\bullet+}$	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J mol^{-1} K^{-1}	$\Delta\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta\Delta S^{\ddagger}$/J mol^{-1} K^{-1}	$T\Delta\Delta S^{\ddagger}$/kJ mol^{-1}
(*S*,*S*)-	12.8 ± 0.9	−152 ± 11			
(*R*,*R*)-	21.7 ± 1.5	−124 ± 8			
			−8.9	−28	−8.3[a)]
	ΔH^{0}/kJ mol^{-1}	ΔS^{0}/J mol^{-1} K^{-1}	$\Delta\Delta H^{0}$/kJ mol^{-1}	$\Delta\Delta S^{0}$/J mol^{-1} K^{-1}	$T\Delta\Delta S^{0}$/kJ mol^{-1}
(*S*,*S*)-	−14.4 ± 1.0	47.3 ± 3.3			
(*R*,*R*)-	−12.8 ± 0.9	51.2 ± 3.6			
			−1.6	−3.9	−1.2[a)]

a) At 25 °C.

Table 5. Kinetic Parameters for the Reactions of cyt *c*(III) and metMb with $BV^{\bullet+}$ at pH 7.0 and $I = 0.040$ M

Protein	Temp/°C	k/10^{8} M^{-1} s^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J mol^{-1} K^{-1}	$T\Delta S^{\ddagger}$/kJ mol^{-1}
cyt *c*(III)	10.0	2.8 ± 0.2			
	15.0	3.3 ± 0.2			
	25.0	4.3 ± 0.3			
	35.0	5.4 ± 0.4			
			16.3 ± 1.1	−25.0 ± 1.8	7.5[a)]
metMb	10.0	0.35 ± 0.03			
	15.0	0.42 ± 0.03			
	25.0	0.49 ± 0.04			
	35.0	0.63 ± 0.05			
			13.5 ± 0.9	−52.1 ± 3.6	15.5[a)]

a) At 25 °C.

with chiral $OAV^{\bullet+}$ was also examined. On the basis of the mechanism in Scheme 1, the association constant (K_1) and the intramolecular ET rate constant (k^{intra}) at 10–35 °C were evaluated using Eq. 3. The numerical data are summarized in Table 2. The activation parameters for the intramolecular ET reaction were obtained from Eyring plots and the enthalpy and entropy changes for the association between metMb and $OAV^{\bullet+}$ were obtained from plots of ln K_1 vs 1/T. The data are summarized in Table 4. The association constant decreases with increasing temperature, arising from a negative ΔH^{0}. Since the entropy change is positive, desolvation may be important for the association between metMb and $OAV^{\bullet+}$. The stereoselectivity for the association process is slightly dependent on the temperatures; a small enthalpy-change difference ($\Delta\Delta H^{0}$) is compensated by the entropy term. On the other hand, the stereoselectivity in the intramolecular ET process is dependent on the temperature; the stereoselectivity increases with decreasing temperature, arising from the enthalpy term. The differences in the enthalpy and entropy changes might be explained by a hydrogen-bonding effect of the CONH groups in the chiral viologens (vide infra). The small stereoselectivity in the present systems may arise because the reactive center in the chiral viologens is far from the chiral centers.

Reactions with Achiral Viologen-Radical Cations. To investigate the role of the CONH group in the chiral viologen-radical cation, we examined the ET reactions of metMb and cyt c(III) with an achiral benzylviologen-radical cation ($BV^{\bullet+}$), although several researchers have investigated the reaction of cyt *c*(III) with $BV^{\bullet+}$.[43,44] The pseudo first-order rate constant is linearly dependent on the concentrations of cyt *c*(III), as shown in Fig. 4. The second-order rate constants and the activation parameters are summarized in Table 5. The second-order rate constant ($k = (4.3 \pm 0.3) \times 10^{8}$ M^{-1} s^{-1}) at 25 °C is in good agreement with the reported data ($k = (4.0–4.3) \times 10^{8}$ M^{-1} s^{-1} at $I = 0.10–0.15$ M).[43,44]

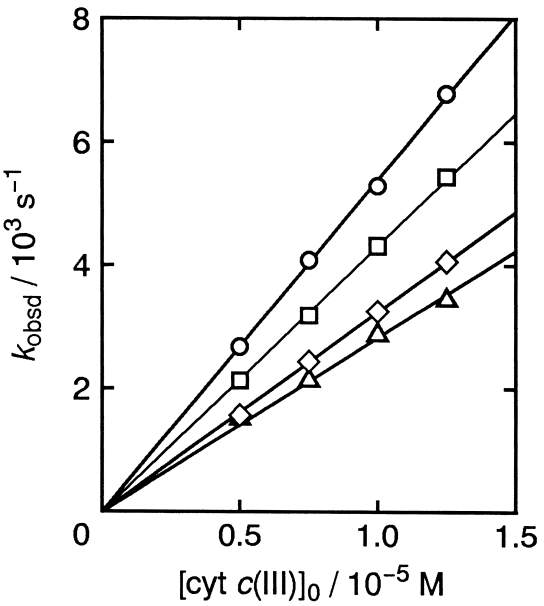


Fig. 4. Plots of k_{obsd} vs [cyt *c*(III)]$_0$ for the reaction of cyt *c*(III) with $BV^{\bullet+}$ at pH 7.0 and $I = 0.040$ M, (△) 10 °C, (◇) 15 °C, (□) 25 °C and (○) 35 °C.

Figure 5 shows the linear dependence of k_{obsd} on the concen-

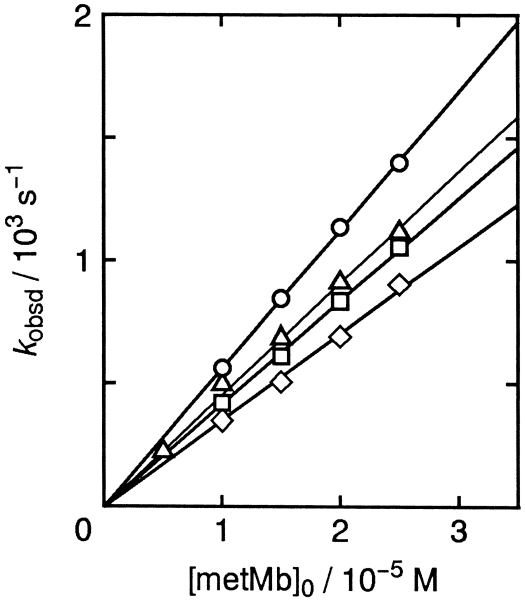


Fig. 5. Plots of k_{obsd} vs [metMb]$_0$ for the reaction of metMb with $BV^{\bullet+}$ at pH 7.0 and I = 0.040 M. (△) 10 °C, (◇) 15 °C, (□) 25 °C, and (○) 35 °C.

trations of metMb for reduction by $BV^{\bullet+}$, indicating no appreciable complex formation between metMb and $BV^{\bullet+}$. The second-order rate constant and the activation parameters are listed in Table 5. It is known that the reaction of metMb with the methylviologen-radical cation has a linear dependence of k_{obsd} on the concentrations of metMb.[45,46] Therefore, the saturation kinetics obtained for the reactions of metMb with the chiral viologen-radical cations might arise from a hydrogen-bonding interaction between the polypeptide side chain in the former and the CONH groups in the latter. The difference in stereoselectivity arises mainly from the enthalpy term, reflecting the hydrogen-bonding interaction. We assume that Lys and His residues near the heme pocket of metMb interact with the CONH group of the chiral viologen-radical cations.

No appreciable interaction of cyt *c*(III) with $OAV^{\bullet+}$ or $CHOAV^{\bullet+}$ might arise from the charge repulsion by the highly positive charge of cyt *c*(III). The values of $\Delta H^{\ddagger}$ for the reduction of cyt *c*(III) by $CHOAV^{\bullet+}$ are lower than that for the achiral $BV^{\bullet+}$. On the other hand, the values of $\Delta H^{\ddagger}$ for $OAV^{\bullet+}$ are similar to that for $BV^{\bullet+}$ and the $\Delta S^{\ddagger}$ values for both $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ are lower than that for $BV^{\bullet+}$. Therefore, the hydrogen-bonding interaction between cyt *c*(III) and the CONH group of $OAV^{\bullet+}$ and $CHOAV^{\bullet+}$ may contribute to the entropy term.

In conclusion, the pseudo first-order rate constant of the decay of the viologen-radical cation in the metMb system was saturated with increasing concentrations of metMb, indicating that metMb interacts with the viologen-radical cation to form a complex, followed by an intramolecular ET reaction. The stereoselectivity was observed in both the complex formation and the intramolecular ET processes for the (*S*,*S*)-isomers of chiral viologens. The saturation kinetics was not observed for the ET reaction between metMb and achiral benzylviologen-radical cation, which has no CONH bond, suggesting that the hydrogen bonding of CONH group of the chiral viologen with the side chain of the polypeptide in metMb, plays an important role in the stereoselectivity. No appreciable complex formation was observed in the ET reaction between cyt *c*(III) and the viologen-radical cations, although the stereoselectivity was observed.

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