CHEMISTRY LETTERS, pp. 2377-2380 1994.

Stereoselective Photoinduced Electron Transfer of Zinc Myoglobin with Optically Active Viologens

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The excited triplet state of zinc myoglobin was preferentially quenched by (*S,S*)-isomers of optically active viologens, 1,1'-bis(1-phenylethylcarbamoylmethyl)-4,4'-bipyridinium (OAV^{2+}) and 1,1'-bis(1-(1-naphthyl)ethylcarbamoylmethyl)-4,4'-bipyridinium ($NOAV^{2+}$) ions: $k_q((S,S)-)/k_q((R,R)-)$ = 1.5 for $NOAV^{2+}$ and 1.3 for OAV^{2+} at 25 °C and an ionic strength of 0.02 mol dm^{-3}.

Stereoselectivity in the electron-transfer (ET) reactions between metalloproteins and chiral metal complexes has been recently reported by using spinach plastocyanin,[1] horse cytochrome *c*,[2] and plant ferredoxin.[3] There is, however, no report on the stereoselectivity in the reactions between metalloprotein and chiral organic reducing agent, especially in the photoinduced ET reaction. 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.[4] They undergo effective one-electron redox reactions with hemoproteins and excited triplet state of zinc-substituted hemoproteins;[5] the stereoselectivity in the photoinduced ET by using viologen must have significant information on the mechanism of ET reactions of myoglobin. We now report on the first example of the stereoselective photoinduced ET reaction between hemoprotein and optically active organic small molecule; (*S,S*)-isomers of optically active viologens (Fig. 1)[6] preferentially quench the excited triplet state of zinc myoglobin (^{*3}ZnMb).

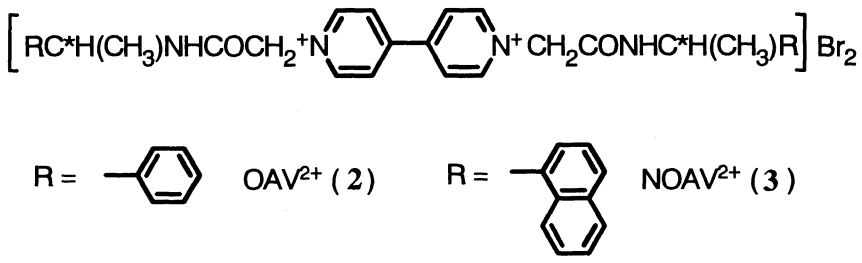

Fig. 1. Structure of optically active viologens.

Optically active viologens, 1,1'-bis(1-phenylethylcarbamoylmethyl)-4,4'-bipyridinium dibromide (OAV^{2+} (**2**)) and 1,1'-bis(1-(1-naphthyl)ethylcarbamoylmethyl)-4,4'-bipyridinium dibromide ($NOAV^{2+}$ (**3**)), were prepared by the method as is shown in Scheme 1 for (*R,R*)- and (*S,S*)-isomers.[7] The (*R,S*)-isomers of OAV^{2+} and $NOAV^{2+}$ were prepared by the reaction of **1** ((*R*)-isomer) with 4,4'-bipyridine in excess, followed by reacting with the (*S*)-isomer of **1** in excess. Bromide salts were con-

$$BrCH_2COOH \xrightarrow[CH_2Cl_2 \text{ at } -5\,^oC]{DCC\,/\,H_2NC^*H(CH_3)R} BrCH_2CONHC^*H(CH_3)R \quad \mathbf{1}$$

$$\mathbf{1} + 4,4'\text{-bpy} \xrightarrow[DMF]{\text{at } 85\,^oC \text{ for } 24\text{ h}} \mathbf{2} \text{ or } \mathbf{3}$$

Scheme 1.

verted to chloride salts by an anion exchange chromatography for photochemical measurements.

The excited triplet state of zinc myoglobin, which was monitored at 460 nm, decayed exponentially with a rate constant of 70 s^{-1} at 25 °C and pH 7.0 in a 0.010 mol dm^{-3} degassed phosphate buffer solution, using a Photal RA-412 pulse flash apparatus with a 30 μs pulse-width Xe lamp (λ > 450 nm).[8] ^{*3}ZnMb was quenched by $NOAV^{2+}$ and OAV^{2+} ions, and the quenching rate was linearly dependent on the concentrations of viologens (Fig. 2).

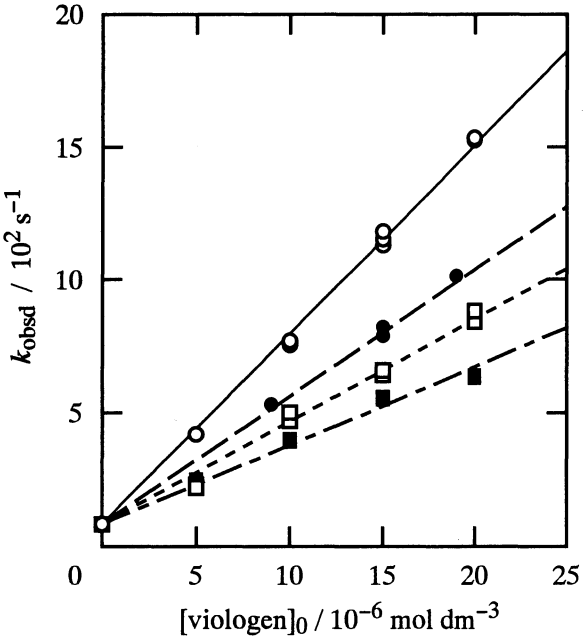

Fig. 2. Plots of k_{obsd} vs. [viologen]$_0$ for the quenching of ^{*3}ZnMb ((0.5–3.0)×10^{-6} mol dm^{-3}) by optically active viologens at 25 °C, pH = 7.0, and I = 0.02 mol dm^{-3}. ○: (*S,S*)-$NOAV^{2+}$, ●: (*R,R*)-$NOAV^{2+}$, □: (*S,S*)-OAV^{2+}, and ■: (*R,R*)-OAV^{2+}.

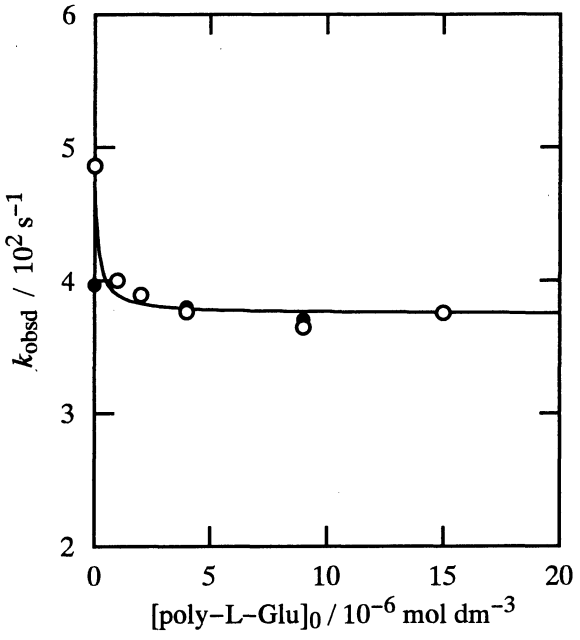

Fig. 3. Effect of poly-L-glutamate on the quenching of ^{*3}ZnMb by OAV^{2+} at [ZnMb]$_0$ = 1.0×10^{-6} mol dm^{-3} and [OAV^{2+}]$_0$ = 1.0×10^{-5} mol dm^{-3}, 25 °C, and I = 0.05 mol dm^{-3}. ○: (*S,S*)-OAV^{2+} and ●: (*R,R*)-OAV^{2+}.

The quenching rate constant (k_q) was obtained from the slope of the plots of k_{obsd} vs. the concentrations of viologens. The values of k_q were (3.9±0.1) × 10^7 dm^3 mol^{-1} s^{-1} ((*S,S*)-OAV^{2+}), (2.9±0.1) × 10^7 dm^3 mol^{-1} s^{-1} ((*R,R*)-OAV^{2+}), (7.1±0.2) × 10^7 dm^3 mol^{-1} s^{-1} ((*S,S*)-$NOAV^{2+}$), and (4.8±0.2) × 10^7 dm^3 mol^{-1} s^{-1} ((*R,R*)-$NOAV^{2+}$) at an ionic strength (I) of 0.02 mol dm^{-3}, respec-

tively. The ratios of $k_q((S,S)-)/k_q((R,R)-)$ are 1.5 for $NOAV^{2+}$ and 1.3 for OAV^{2+} systems, respectively. Therefore, the (*S,S*)-isomers preferentially quench ^{*3}ZnMb. For the corresponding (*R,S*)-isomers, k_q values are between those for (*R,R*)- and (*S,S*)-isomers. At higher ionic strength of 0.32 mol dm^{-3} (NaCl), the ratios of $k_q((S,S)-)/k_q((R,R)-)$ became smaller: 1.4 for $NOAV^{2+}$ ((9.3±0.3) × 10^7 dm^3 mol^{-1} s^{-1} ((*S,S*)-) and (6.6±0.3) × 10^7 dm^3 mol^{-1} s^{-1} ((*R,R*)-)) and 1.0 for OAV^{2+} ((4.9 ±0.1) × 10^7 dm^3 mol^{-1} s^{-1} for both (*S,S*)- and (*R,R*)-isomers), although high stereoselectivity is still held for $NOAV^{2+}$.

The quenching of ^{*3}ZnMb has been assumed to occur by a conformational gating mechanism.[5] The polypeptide chain of myoglobin has an *S*-configuration and the (*S,S*)-isomer of viologen might be more fitted 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 very similar: −0.20 V vs. NHE at 25 °C in aqueous 0.1 mol dm^{-3} KCl solutions. 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 the steric acceleration takes place in this system.

We also examined the effect of optically active poly-L-glutamate ions on the quenching of ^{*3}ZnMb by OAV^{2+} (Fig. 3). The quenching rate decreased with an increase in the concentrations of sodium poly-L-glutamate (mean F.W. = 13,000) and became constant at $[\text{poly-L-Glu}]_0 \geq 9 \times 10^{-6}$ mol dm^{-3}, where no stereoselectivity was observed. The solid curve is fitted to the equation, $k_{obsd} = k_0 + (k_q + k_q^{complex}K[\text{poly-L-Glu}]_0)[OAV^{2+}]_0 / (1 + K[\text{poly-L-Glu}]_0)$, where k_0 (= 70 s^{-1}) is the rate constant of the spontaneous decay, k_q (= 3.9 × 10^7 dm^3 mol^{-1} s^{-1}) and $k_q^{complex}$ (= 2.8 × 10^7 dm^3 mol^{-1} s^{-1}) are the quenching rate constants by free (*S,S*)-OAV^{2+} and (*S,S*)-OAV^{2+} bound to poly-L-glutamate, respectively, and *K* (= 5.9 × 10^4 dm^3 mol^{-1}) is the association constant between OAV^{2+} and poly-L-glutamate. We also found from the spectrophotometric measurements that (*S,S*)-OAV^{2+} binds poly-L-glutamate ion more strongly than (*R,R*)-OAV^{2+}; there is stereoselectivity in the association between OAV^{2+} and poly-L-glutamate, the latter having an *S*-configuration. The binding constants (*K*) are (5.9±0.6) × 10^4 dm^3 mol^{-1} for (*S,S*)-OAV^{2+} and (4.3±0.5) × 10^4 dm^3 mol^{-1} for (*R,R*)-OAV^{2+} at 25 °C and *I* = 0.03 mol dm^{-3} (NaCl), respectively. The *K* value (5.9 × 10^4 dm^3 mol^{-1}) obtained from the fitting curve in Fig. 3 is in agreement with that obtained from spectrophotometric measurements. The formation of the complex between OAV^{2+} and poly-L-glutamate decreases the quenching rate of ^{*3}ZnMb due to an increase in the separation distance of the redox partners. The rate constant of the quenching of ^{*3}ZnMb by (*S,S*)-OAV^{2+} bound to poly-L-glutamate is 2.8 × 10^7 dm^3 mol^{-1} s^{-1}, which is smaller than that for the free (*S,S*)-OAV^{2+} ion (3.9 × 10^7 dm^3 mol^{-1} s^{-1}).

This research was partly supported by a Grant-in-Aid for Scientific Research No. 06640724 from the Ministry of Education, Science and Culture.

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6) Rau and Ratz have first reported the stereoselectivity in the luminescence quenching of chiral $^{*3}[Ru(bpy)_3]^{2+}$ by optically active viologen, 1-methyl-1'-[(3*S*)-(-)-3-pinamylmethyl)-4,4'-bipyridinium dichloride, which was, however, not optically pure. See: H. Rau and R. Ratz, *Angew. Chem. Int. Ed. Engl.*, **22**, 550 (1983).

7) Crude products were recrystallized twice from methanol and the total yields were 12-19%. All the compounds **2** and **3** ((*R,R*)-, (*S,S*)-, and (*R,S*)-isomers) gave satisfactory elemental analyses. **2**: UV (H_2O), λ_{max} 264 nm (log ε 4.33); ^1H NMR (270 MHz, D_2O, DSS) δ 1.50 (6H, d, *J* = 6.6 Hz, CH_3), 4.94 (2H, m, CH), 5.59 (4H, d, *J* = 4.9 Hz, CH_2), 7.32-7.40 (10H, m, C_6H_5), 8.54 (4H, d, *J* = 6.4 Hz, 3,5-bpy), and 8.98 (4H, d, *J* = 6.4 Hz, 2,6-bpy); ORD (*c* 0.025, H_2O, 20 °C) $[\Phi]_{589}$ +176.0° for (*R,R*)-, $[\Phi]_{589}$ -175.0° for (*S,S*)-, and $[\Phi]_{589}$ 0° for (*R,S*)-isomer. **3**: UV (H_2O), λ_{sh} 260 nm (log ε 4.43), λ_{max} 271 nm (log ε 4.49), λ_{max} 278 nm (log ε 4.47), and λ_{sh} 290 nm (log ε 4.28); ^1H NMR (270 MHz, 1.0 × 10^{-3} mol dm^{-3} in D_2O, DSS) δ 1.64 (6H, d, *J* = 6.8 Hz, CH_3), 5.58 (4H, d, *J* = 5.9 Hz, CH_2), 5.77 (2H, m, CH), 7.50-7.60 (6H, m, 3,6,7-naphthyl), 7.66 (2H, d, *J* = 6.8 Hz, 2-naphthyl), 7.88 (2H, d, *J* = 7.3 Hz, 4-naphthyl), 7.95 (2H, d, *J* = 7.3 Hz, 5-naphthyl), 8.11 (2H, d, *J* = 7.3 Hz, 8-naphthyl), 8.51 (4H, d, *J* = 7.3 Hz, 3,5-bpy), and 8.97 (4H, d, *J* = 6.8 Hz, 2,6-bpy); ORD (*c* 0.050, H_2O, 20 °C) $[\Phi]_{589}$ +77.7° for (*R,R*)-, $[\Phi]_{589}$ -78.4° for (*S,S*)-, and $[\Phi]_{589}$ 0° for (*R,S*)-isomer. ^1H NMR spectra of **3** were dependent on the concentrations, arising from the self-association of $NOAV^{2+}$ which exists in a monomer less than 2.0 × 10^{-3} mol dm^{-3}. Detailed results will be published elsewhere.

8) When the aqueous solution of $NOAV^{2+}$ was irradiated by UV light, a fluorescence of naphthyl groups was intramolecularly quenched (λ_{em} = 330 nm and τ = 0.5 ns; τ_0 = 16 ns for sodium naphthalene-1-sulfonate as a reference). The quenching reaction of the excited singlet state of $NOAV^{2+}$ does not contribute to the present ^{*3}ZnMb system.

(Received September 9, 1994)