Stereoselective Electron-Transfer Reaction between Metmyoglobin and Chiral Viologen-Radical Cation through Pre-Complexation

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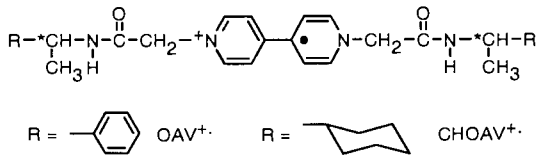
Metmyoglobin was reduced by chiral viologen-radical cations containing ((1-phenyl- or ((1-cyclohexylethyl)carbamoyl)-methyl group through pre-complexation followed by intracomplex electron transfer; (*S,S*)-isomers of chiral viologens showed slightly higher association constants as well as larger intracomplex electron-transfer rates.

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 electron-transfer (ET) reactions between metalloproteins and chiral small molecules has been reported.[1] We have recently reported the stereoselective ET quenching of the excited triplet state of zinc-substituted myoglobin (^{3}(ZnMb)*) by chiral viologens, where there is no evidence for the complex formation between ^{3}(ZnMb)* and viologens.[2] This may arise partly because the 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. In this work we report that metmyoglobin (metMb) interacts with chiral viologen-radical cations ($OAV^{+\cdot}$ and $CHOAV^{+\cdot}$) to form a complex followed by an intracomplex ET reaction.



The radical cations, $OAV^{+\cdot}$ and $CHOAV^{+\cdot}$, were prepared in situ by the photochemical reduction of the parent viologens[2] 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. Under the present experimental conditions, ^{3}([Ru(bpy)$_3$]$^{2+}$)* reacted only with viologen to produce the (2.0—5.0) × 10^{-7} M radical cation (M = mol dm^{-3}), which then reacted with horse heart metMb. In the absence of metMb only the formation of viologen-radical cation was observed. The formation of deoxyMb and the decays of metMb and the viologen-radical cation were followed spectrophotometrically, after flashing the solution with a Xe pulse flash lamp (a Photal RA 412 apparatus). The experimental conditions are as follows: [metMb]$_0$ = (0.30 — 3.00) × 10^{-5} M, [viologen^{2+}]$_0$ = 1.00 × 10^{-3} M, [[Ru(bpy)$_3$]$^{2+}$]$_0$ = 1.00 × 10^{-5} M, and [Na_2H_2-edta]$_0$ = 5.00 × 10^{-3} M at 25.0 °C, pH 7.0 (a 0.020 M phosphate buffer), and an ionic strength (I) of 0.040—0.50 M (NaCl).

Figure 1 shows the absorption spectral changes after irradiation by light of the degassed solution containing metMb,

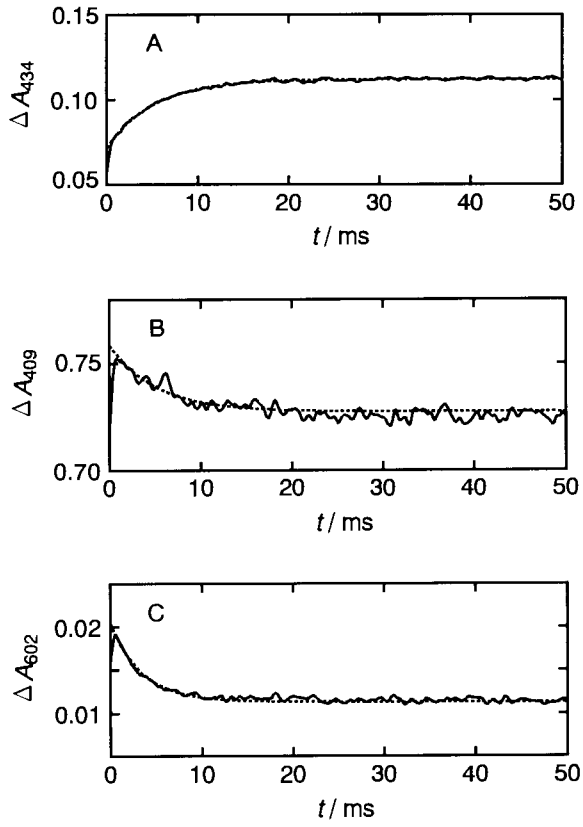


Figure 1. Absorption spectral changes for the reaction of metMb (2.00 × 10^{-5} M) with $CHOAV^{+\cdot}$ at 25.0 °C, pH 7.0, and I = 0.50 M (NaCl). (A) Formation of deoxyMb at 434 nm. (B) Decay of metMb at 409 nm. (C) Decay of (*R,R*)-$CHOAV^{+\cdot}$ at 602 nm. Dotted lines are fitted to the first-order kinetics.

$CHOAV^{+\cdot}$, [Ru(bpy)$_3$]$^{2+}$, and Na_2H_2edta at 25.0 °C, pH 7.0 (0.020 M phosphate buffer), and I = 0.50 M (NaCl). 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 $CHOAV^{+\cdot}$. The observed first-order rate constant (k_{obsd}) was saturated with increasing the concentrations of metMb at variety of ionic strengths (Figure 2). The reaction rate increased with increasing ionic strengths, indicating that the reactive species are positively charged.[3,4] It is, therefore, demonstrated that metMb interacts with the viologen-radical cation to form a complex followed by an *intra*complex ET reaction,

$$\text{metMb} + \text{OAV}^{+\cdot} \overset{K}{\rightleftharpoons} \{\text{metMb} \cdots \text{OAV}^{+\cdot}\} \xrightarrow{k^{intra}} \{\text{deoxyMb} \cdots \text{OAV}^{2+}\} \xrightarrow{fast} \text{deoxyMb} + \text{OAV}^{2+}$$

From the above mechanism, k_{obsd} is represented by the following

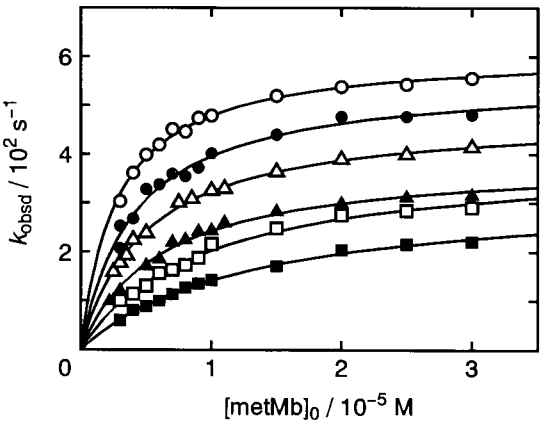


Figure 2. Plots of k_{obsd} vs. [metMb]$_0$ for the reaction of metMb (2.00×10^{-5} M) with OAV$^{+\cdot}$ at 25.0 °C and pH 7.0. Open symbols, (*S,S*)-OAV$^{+\cdot}$ and closed symbols, (*R,R*)-OAV$^{+\cdot}$. (□, ■) I = 0.040 M, (△, ▲) I = 0.10 M, and (○, ●) I = 0.50 M.

equation,

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

Here [metMb]$_0$ is the initial concentration of metMb. The values of k^{intra} and K are listed in Table 1. There is stereoselectivity in both k^{intra} and K for the (*S,S*)-isomers, although the stereoselectivity is small: 1.1—1.4 for k^{intra} and 1.2—1.5 for K.

It has been reported that metMb is reduced by methylviologen-radical cation (MV$^{+\cdot}$) without any complex formation; a linear dependence of k_{obsd} vs. the concentrations of metMb was observed ($k = 5.4 \times 10^7$ M^{-1} s^{-1} at 25 °C and I = 0.2 M).[5] We also examined the reaction of metMb with a 1,1'-dibenzyl-4,4'-bipyridinium radical cation (BV$^{+\cdot}$) and the same linear dependence of k_{obsd} on [BV$^{+\cdot}$]$_0$ was observed ($k = 5.1 \times 10^7$ M^{-1} s^{-1} at 25 °C and I = 0.10 M). Thus, the high k_{obsd} value and the saturation behaviors are unique for OAV$^{+\cdot}$ and CHOAV$^{+\cdot}$. It is interesting that the reactivities of OAV$^{+\cdot}$ and CHOAV$^{+\cdot}$ are similar to each other. It is, therefore, considered that the aromatic group of the chiral substituent is not important in the complexation but instead the carbamoyl group may have an important role. A possible explanation may be that the carbamoyl groups interact with carboxylate and/or ammonium groups of metMb through hydrogen bonding. In the preliminary experiment of the reduction of cytochrome *c*, we have found the linear dependence of k_{obsd} vs. the concentrations of cytochrome *c* which is a basic protein having a formal charge of +7.[6] The charge of the surface of the protein might be important to control the complex formation.

Table 1. Intracomplex electron-transfer rate constants and association constants for the reaction of metMb with viologen-radical cation at 25.0 °C and pH 7.0 (0.020 M phosphate buffer)

Viologen radical	I/M	$k^{intra}/10^2$ s^{-1} (*S,S*)-	(*R,R*)-	$K/10^5$ M^{-1} (*S,S*)-	(*R,R*)-
OAV$^{+\cdot}$	0.040	4.0±0.2	3.3±0.2	1.1±0.1	0.78±0.05
	0.10	4.8±0.3	3.9±0.3	2.0±0.1	1.7±0.1
	0.50	6.1±0.3	6.0±0.3	3.6±0.2	2.4±0.1
CHOAV$^{+\cdot}$	0.10	3.0±0.2	2.2±0.1	1.2±0.1	0.94±0.05
	0.50	4.1±0.2	3.9±0.2	1.5±0.1	1.0±0.1

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