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Kinetics and Mechanisms of Photoinduced Electron-Transfer Reaction of Magnesium Myoglobin

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Horse heart metmyoglobin was reconstituted with magnesium(II) porphyrin, [Mg(dp)] (3,7,12,17-tetramethyl-2,18-bis(2-carboxyethyl)porphyrinatomagnesium(II)). Photoinduced electron-transfer (ET) between magnesium myoglobin (MgDPMb) and cationic and anionic quenchers, such as viologens and anthraquinone-2-sulfonate (AQS^-), has been studied in an aqueous degassed solution. None of the quenchers examined in this work formed a detectable associated complex with MgDPMb. The thermal backward ET reaction was observed for these quenchers. Not only photoinduced ET but also thermal backward ET reactions were insensitive to the driving force of the reactions. Moreover, there was little effect of metal-ion substitution; the rate of reaction was similar to that for zinc myoglobin. Thus, the reactions are controlled by conformational changes in the myoglobins.

Photoinduced electron-transfer (ET) reactions of metalloporphyrins have received considerable attention in the fields of both chemistry and biochemistry. Reconstituted hemoproteins with zinc and magnesium ions, such as zinc cytochrome *c* and zinc- and magnesium myoglobins, are very important for studying the role of apoprotein in the photoinduced ET reaction of biological systems, because the lifetime of these metal substituted hemoproteins is much longer than that of free metalloporphyrin in solution.1—10) ET reaction theories such as the Marcus theory have been applied to many ET reactions involving metalloproteins;11) a number of systems have shown the driving force dependence on the ET rate constant. However, another factor controlling the ET rate, conformational changes (a gating mechanism),12) has been proposed in the ET reactions of zinc-cytochrome *c*,13) hemoglobin,8) and myoglobin.6) We have recently reported that not only photoinduced ET but also thermal backward ET reactions of zinc myoglobin were insensitive to the driving force of the reactions and that the reactions might be controlled by conformational changes in zinc myoglobin.10,14) We expect that the conformational change will not be affected by metal-ion substitution of zinc myoglobin, since the coordination geometry of the central metal ion of myoglobin does not change. It has been reported that magnesium myoglobin has a five coordinate geometry, like that of zinc myoglobin.5) Very few studies on the photoinduced ET reaction of magnesium myoglobin have been reported. In the present study, we shall check the gating mechanism by metal-ion substitution of myoglobin with a magnesium(II) ion; magnesium(II) porphyrin is a useful prosthetic group for monitoring the photoinduced reaction in biomimic systems.

Experimental

Reagents. Metmyoglobin from horse heart muscle was obtained from Sigma and was purified as previously described.15,16) 3,7,12,17-Tetramethyl-2,18-bis(2-carboxyethyl)porphyrinatomagnesium(II) ([Mg(dp)]) was prepared by a previously reported method for [Mg(mp)] (H_2mp=8,13-diethyl-3,7,12,17-tetramethyl-2,18-bis(2-carboxyethyl)porphyrin).5) Recombination of [Mg(dp)] with apomyoglobin was carried out at 4 °C in the dark using a method that has already been published for zinc myoglobin (ZnPPMb, PP=H_2pp=3,7,12,17-tetramethyl-8,13-divinyl-2,18-bis(2-carboxyethyl)porphyrin).2—5) Although molar absorption coefficients for MgDPMb have not been reported, the concentrations of MgDPMb were estimated by assuming that the molar absorption coefficients are the same as those for [Mg(dp)] in pyridine solution ($\varepsilon_{409}=2.9\times10^5$ $M^{-1}\,cm^{-1}$, 1 M=1 $mol\,dm^{-3}$). This value is similar to that for MgPPMb ($\varepsilon_{421}=3.0\times10^5$ $M^{-1}\,cm^{-1}$).17) The MgDPMb solution, whose absorption ratio of A_{409}/A_{278} is 20, was used for kinetic measurements. Perchlorate salts of 1,1′-dimethyl-4,4′-bipyridinium (MV^{2+})18) and 1-methyl-1′-carboxymethyl-4,4′-bipyridinium (CMV^+)19) ions and chloride salts of 1,1′-ethylene-2,2′-bipyridinium (DQ^{2+}) and 1,1′-tetramethylene-4,4′-dimethyl-2,2′-bipyridinium ($MPDQ^{2+}$) ions20) were prepared by a previously reported method. All other chemicals used were of guaranteed grade from Wako Pure Chemical Industries, Ltd. All of the solutions were prepared with 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_{409}/A_{278} was checked for each solution. A single flash photolysis was carried out in the degassed solutions containing MgDPMb ($(0.3—3.0)\times10^{-6}$ M) and quenchers ($(0.25—4.00)\times10^{-5}$ M) at 25 °C and pH 7.0 (a 0.01 M phosphate buffer) 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). Absorbance changes during the reaction were monitored at 410, 438, and 680 nm. The lifetime of the fluorescence of MgDPMb was measured using a Horiba NAES-500 nanosecond fluorometer. Fluorescence spectra were measured using a Hitachi-850 spectrofluorometer. 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 and Discussion

Excited State of MgDPMb. The fluorescence spectrum of MgDPMb on excitation at 542 nm (emission wavelengths are 585 and 638 nm) in a degassed aqueous solution at pH 7.0 (a 0.01 M phosphate buffer) is similar to that of MgPPMb (592 nm and 652 nm)[17] or MgMPMb (585 nm and 638 nm).[5] The fluorescence lifetime of MgDPMb was 11.4±0.4 ns (the relative amplitude of 0.98, $\chi^2=1.29$) with a minor component (less than 1 ns with a relative amplitude of 0.02). The lifetime of MgPPMb is 10.0 ns (the relative amplitude of 0.89);[17] there is a contribution of the short component (1.1 ns with a relative amplitude of 0.11), the origin of which is not known. The triplet–triplet absorption spectral maxima of MgDPMb were 438, 630, and 680 nm; the absorptions at the latter two wavelengths were very weak. The lifetime of the excited triplet state of MgDPMb determined at 438 nm was 13 ms at 25 °C and was shorter than that for MgPPMb (42 ms)[2] or MgMPMb (33 ms).[5] This is probably due to the lack of substituents at the 8- and 13-positions on the porphyrin; it is likely that [Mg(dp)] is less stable than [Mg(pp)] in the heme pocket of myoglobin.

Intermolecular ET Quenching. The excited singlet state of MgDPMb was not quenched by the quenchers used in this work; the lifetime did not change in the presence of the quenchers. However, the excited triplet state of MgDPMb was efficiently quenched by them. One of the examples is shown in Fig. 1. The absorbance changes at 410 and 680 nm were biphasic for all of the quencher systems examined in this work. The absorptions of the ground state of MgDPMb and of its radical cation ($\mathrm{MgDPMb^+}$) are predominant at 410 nm. The decay of *MgDPMb monitored at 438 nm, where the absorption of *MgDPMb is predominant, was monophasic and first order for at least 85% of the reaction. Therefore, the first-order rate constant was evaluated at 438 nm for the quenching reaction. The values of k_{obsd} were linearly dependent on the concentrations of the quenchers, as is shown in Fig. 2. The formation and decay of $\mathrm{MgDPMb^+}$ were monitored at 680 nm, where the absorption of $\mathrm{MgDPMb^+}$ is predominant (see the bottom of Fig. 1). The appearance and decay of viologen radical cations were also observed at 600 nm and the absorbance change was less than that observed at 680 nm for $\mathrm{MgDPMb^+}$. The second-order plots for the decay of $\mathrm{MgDPMb^+}$ are shown in Fig. 3. The second-order rate constants were evaluated from the latter linear portion of the plots, at which portion the formation of $\mathrm{MgDPMb^+}$ does not interfere with the decay process. It was found that flash illumination of MgDPMb yields about 60% triplet state of MgDPMb, on the basis of the absorbance change at 410 nm ($\Delta\varepsilon=\varepsilon(\text{ground})-\varepsilon(\text{triplet})=1.9\times10^5\ \mathrm{M^{-1}\,cm^{-1}}$). The concentrations of $\mathrm{MgDPMb^+}$ formed at the first stage were estimated to be 60% of the initial concentrations of MgDPMb. Therefore, the rate constant for the slow reaction (k_b in Eq. 3) was obtained form (slope)×(molar absorption coefficient of $\mathrm{MgDPMb^+}$).

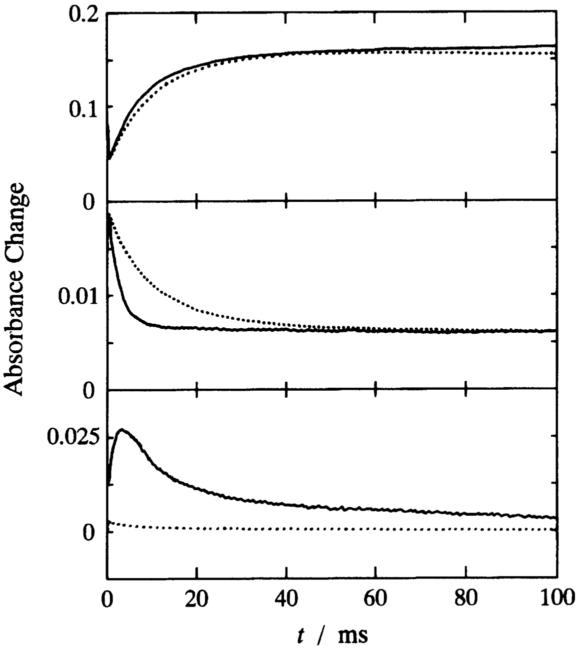

Fig. 1. Absorbance changes following production of *MgDPMb by irradiation of MgDPMb (1.0×10^{-6} M) with a Xe-flash lamp in the presence of $\mathrm{CMV^+}$ (1.0×10^{-5} M) at 25 °C, pH 7.0 (a 0.01 M phosphate buffer), and $I=0.02$ M. Top: 410 nm, Middle: 438 nm, and Bottom: 680 nm. The dotted lines are those in the absence of quenchers.

The biphasic behavior, fast and slow reactions, can be attributed to the formation of the radical ions ($\mathrm{MgDPMb^+}$ and $\mathrm{Q^-}$) and the thermal backward ET reaction, respectively. The photoinduced ET reactions of *MgDPMb can be represented by the following equations:

$$\mathrm{MgDPMb} \underset{k_0}{\overset{h\nu}{\rightleftarrows}} {}^*\mathrm{MgDPMb}, \tag{1}$$

$$ {}^*\mathrm{MgDPMb} + \mathrm{Q} \xrightarrow{k_\mathrm{q}} \mathrm{MgDPMb^+} + \mathrm{Q^-}, \tag{2}$$

and

$$\mathrm{MgDPMb^+} + \mathrm{Q^-} \xrightarrow{k_\mathrm{b}} \mathrm{MgDPMb} + \mathrm{Q}. \tag{3}$$

The decay of *MgDPMb and the formation of $\mathrm{MgDPMb^+}$ obey the following rate laws:

$$-\,\mathrm{d}[{}^*\mathrm{MgDPMb}]/\mathrm{d}t = (k_0 + k_\mathrm{q}[\mathrm{Q}])[{}^*\mathrm{MgDPMb}] \tag{4}$$

and

$$\mathrm{d}[\mathrm{MgDPMb^+}]/\mathrm{d}t = k_\mathrm{q}[{}^*\mathrm{MgDPMb}][\mathrm{Q}] - k_\mathrm{b}[\mathrm{MgDPMb^+}][\mathrm{Q^-}]. \tag{5}$$

The rate constants for both photoinduced ET and thermal backward ET reactions (k_q and k_b) are listed in Table 1, along with the data for ZnPPMb.

Mechanism of Reaction. Both the k_q and k_b values listed in Table 1 are in a narrow range for a variety

Table 1. Rate Constants for the Quenching and Thermal Backward ET Reactions of MgDPMb and ZnPPMb at 25 °C, pH 7.0, and I=0.02 M (a 0.01 M Phosphate Buffer)

		$k_q/M^{-1}s^{-1}$		$k_b/M^{-1}s^{-1}$	
Quencher	E^0/V	MgDPMb	ZnPPMb[a]	MgDPMb	ZnPPMb[a]
DQ^{2+}	−0.35	$(6.0\pm0.4)\times10^7$	—	$(4.9\pm0.4)\times10^7$	—
AQS^-	−0.38	$(2.6\pm0.1)\times10^8$	$(2.9\pm0.3)\times10^8$ [b]	$(1.3\pm0.2)\times10^8$	—
CMV^+	−0.42	$(3.2\pm0.2)\times10^7$	$(2.6\pm0.2)\times10^7$	$(9.6\pm1.0)\times10^7$	$(1.1\pm0.2)\times10^8$
MV^{2+}	−0.45	$(2.3\pm0.1)\times10^7$	$(4.7\pm0.5)\times10^7$	$(1.5\pm0.2)\times10^8$	$(4.0\pm1.0)\times10^7$
$MPDQ^{2+}$	−0.69	$(3.2\pm0.2)\times10^7$	—	$(3.6\pm0.3)\times10^8$	—

a) Refs. 10 and 14. b) I=0.05 M. Ref. 3.

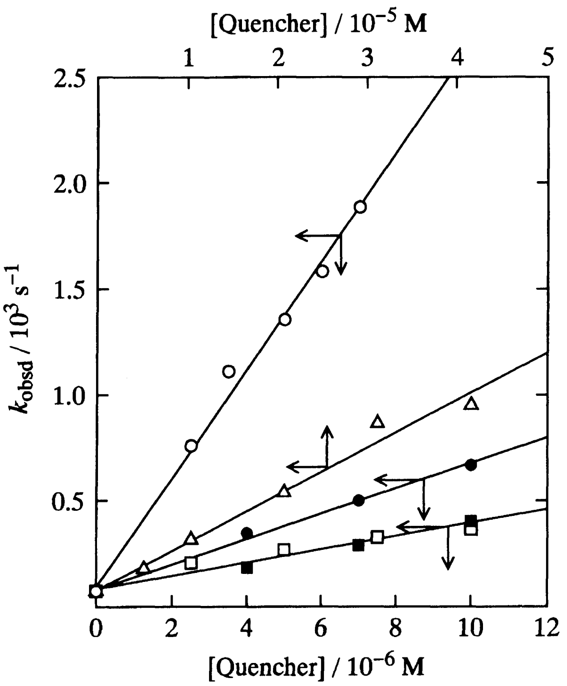

Fig. 2. Plots of k_{obsd} vs. the initial concentrations of quenchers for the quenching of *MgDPMb at 25 °C, $[MgDPMb]_0=(0.3—3.0)\times10^{-6}$ M, and pH 7.0 (a 0.01 M phosphate buffer). ○: AQS^-, ●: DQ^{2+}, □: CMV^+, ■: $MPDQ^{2+}$, and △: MV^{2+}.

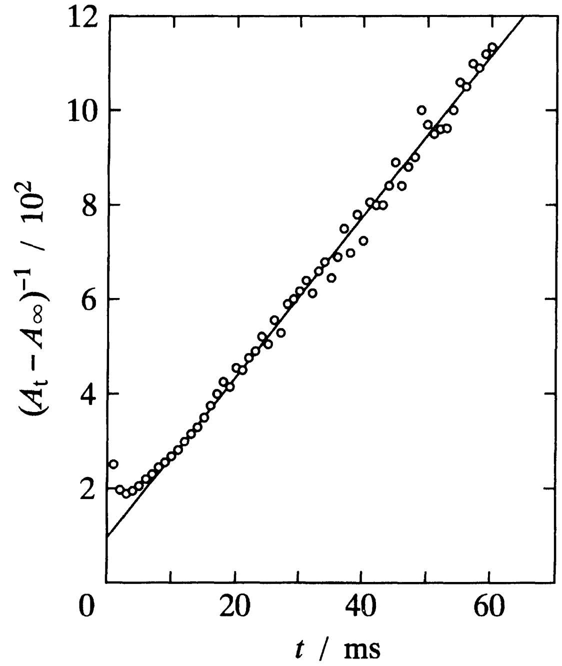

Fig. 3. Plots of $(A_t-A_\infty)^{-1}$ vs. t for the slow phase of the quenching of *MgDPMb by CMV^+. The experimental conditions are the same as in Fig. 1.

of quenchers (their redox potentials span over the range of 0.34 V) and are smaller than the diffusion-controlled limit in aqueous solutions. Moreover, the rate constants for MgDPMb are very close to those for ZnPPMb; there is little effect of the substitution of metal ions.

The Marcus theory has been well applied to metmyoglobin/deoxymyoglobin systems with a variety of redox reagents.[21] Based on the Marcus theory, we calculated the self-exchange rate constants for $MgDPMb^{+/*}$ and $MgDPMb^{+/0}$ systems and obtained a variety of the estimated k_{22}. The value of k_{22} ranges from 1.5×10 $M^{-1}s^{-1}$ (DQ^{2+}) to 2.5×10^5 $M^{-1}s^{-1}$ ($MPDQ^{2+}$) for the former and from 2.0×10^{-11} $M^{-1}s^{-1}$ ($MPDQ^{2+}$) to 3.3×10^{-9} $M^{-1}s^{-1}$ (AQS^-) for the latter, respectively;[22] the application of the Marcus theory was unsuccessful for both quenching and thermal backward ET reactions of MgDPMb. Similar results have been obtained for *ZnPPMb and $ZnPPMb^+$ systems.[10,14] The estimated self-exchange rate constants for the $ZnPPMb^{+/*}$ and $ZnPPMb^{+/0}$ systems were spread out over the ranges of 10^{-6}—10^4 $M^{-1}s^{-1}$ and 10^{-11}—10^{-2} $M^{-1}s^{-1}$, respectively. The variety of the estimated self-exchange rate constants suggests that the quenching of *MgDPMb is not controlled by the ET step or that the ET quenching is nonadiabatic. The latter case is unlikely for these systems since (i) the ET quenching and thermal backward ET reactions efficiently occur in the associated complex of ZnPPMb with a hexacyanoferrate(III) ion[7,14] and in covalently attached pentaammineruthenium(III)-*ZnPPMb systems[24] and (ii) the thermal ET reactions of metmyoglobins with a variety of reductants are adiabatic.[21]

Barboy and Feitelson have proposed a conformational gating mechanism for the bimolecular quenching reaction of *ZnPPMb with AQS^-, MV^{2+}, and O_2.[3] We have supported the gating mechanism for both

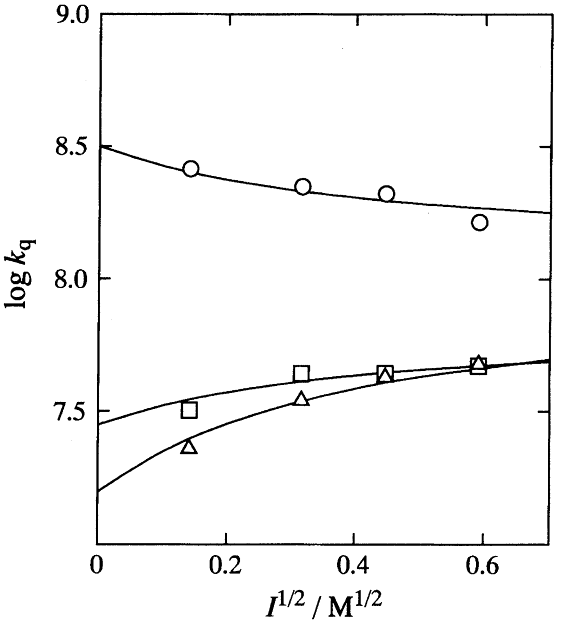

Fig. 4. Plots of $\log k_q$ vs. the square root of ionic strengths for the quenching of *MgDPMb at 25 °C and pH 7.0 (a 0.01 M phosphate buffer). ○: AQS^-, □: CMV^+, and △: MV^{2+}.

ET quenching and thermal backward ET reactions of *ZnPPMb with a variety of quenchers.[10,14)] The present results for MgDPMb also support the mechanism proposed for ZnPPMb:

$$\text{MgDPMb} \underset{k_0}{\overset{h\nu}{\rightleftharpoons}} {}^*(\text{MgDPMb})_A, \quad (6)$$

$$^*(\text{MgDPMb})_A + Q \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} {}^*(\text{MgDPMb})_B + Q, \quad (7)$$

$$^*(\text{MgDPMb})_B + Q \xrightarrow{k_8} (\text{MgDPMb}^+)_B + Q^-, \quad (8)$$

$$(\text{MgDPMb}^+)_B + Q^- \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} (\text{MgDPMb}^+)_A + Q^-, \quad (9)$$

and

$$(\text{MgDPMb}^+)_A + Q^- \xrightarrow{k_{10}} \text{MgDPMb} + Q. \quad (10)$$

Reaction 7 is a conformational change from inactive 'form A' to active 'from B'. The quenching rate is dependent on the concentrations of Q. Therefore, the formation of the active 'form B' is induced by the interaction of Q with myoglobin.[25)] From a steady-state assumption for active from $^*(\text{MgDPMb})_B$, the overall rate constant, k_q, is represented as $k_7k_8/(k_{-7}+k_8)$. When the ET step (Eq. 8) is much faster than the reverse reaction of Eq. 7 ($k_8 \gg k_{-7}$), k_q corresponds to k_7. The thermal backward ET reaction may be also controlled by a conformational change of MgDPMb^+ (Eq. 9), where k_b is represented as $k_9k_{10}/(k_{-9}+k_{10})$ and, when $k_{10} \gg k_{-9}$, k_b becomes k_9.

Ionic Strength Effect. The ionic strength effect on the quenching reaction of *MgDPMb with MV^{2+}, CMV^+, and AQS^- was examined. Figure 4 shows the plots of $\log k_q$ vs. the square root of ionic strengths. We tried to estimate the ion size parameter and the effective charge of *MgDPMb by using the following equation for the ionic strength dependence of the rate constant:

$$\log k_q = \log k_0' + (1.02 z_1 z_2 I^{1/2})/(1 + 3.29 d I^{1/2}), \quad (11)$$

where z_1 and z_2 are the charges of *MgDPMb and Q, respectively, and d (in nm) is the center to center distance of the closest approach of the reactants. The estimated charge of *MgDPMb was +0.7—+0.9 and the distance of the closest approach was about 0.7 nm for these systems. We think that the reactive site of *MgDPMb is the positively charged amino acid residue(s), Lys and/or Arg ($pK_a \approx 10$ for Lys and ≈ 12.5 for Arg in metmyoglobin),[26)] near the heme pocket, the former being predominant on the surface of the protein. A similar effective charge of the reactive site has been estimated for *ZnPPMb (+0.5).[10,14)] The small d value for the reaction of *MgDPMb suggests that the reaction occurs on the surface of the protein, that is, the interaction of Q with *MgDPMb induces a conformational change, followed by a rapid ET reaction (Eq. 8).

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22) The self-exchange rate constant was calculated from the Marcus equation:[23] $k_{12}=(k_{11}k_{22}f_{12}K_{12})^{1/2}$ and $\ln f_{12}=(\ln K_{12})^2/4\ln(k_{11}k_{22}/10^{22})$, by using $k_{11}=1\times10^{8}\ \mathrm{M^{-1}\,s^{-1}}$ (quenchers),[14] $E^\circ=-0.79$ V ($\mathrm{MgDPMb^{+/*}}$),[5] and $E^0=$ 0.93 V ($\mathrm{MgDPMb^{+/0}}$).[5]

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25) One of referees pointed out that Eqs. 7 and 8 may contain the following elementary steps including a preequilibrium of weak binding of Q with $^*(\mathrm{MgDPMb})_\mathrm{A}$:

$$^*(\mathrm{MgDPMb})_\mathrm{A} + \mathrm{Q} \rightleftarrows {}^*(\mathrm{MgDPMb})_\mathrm{A}\cdots\mathrm{Q},$$

$$^*(\mathrm{MgDPMb})_\mathrm{A}\cdots\mathrm{Q} \longrightarrow {}^*(\mathrm{MgDPMb})_\mathrm{B} + \mathrm{Q},$$

and

$$^*(\mathrm{MgDPMb})_\mathrm{B} + \mathrm{Q} \longrightarrow (\mathrm{MgDPMb}^+)_\mathrm{B} + \mathrm{Q}^-. \quad \text{fast}$$

Similar elementary steps may be also contained in the backward ET reaction (Eqs. 9 and 10). In this case the ionic-strength dependence must be realized for each elementary step. Therefore, the estimated values for the charge of MgDPMb and the ion size parameter (*d*) may not be real ones.

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