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# MAGNETIC FIELD EFFECTS ON RADICAL PAIR INTERMEDIATES IN BACTERIAL PHOTOSYNTHESIS

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## SUMMARY

We have investigated the effects of magnetic fields on the formation and decay of excited states in the photochemical reaction centers of *Rhodopseudomonas sphaeroides*. In chemically reduced reaction centers, a magnetic field decreases the fraction of the transient state $P^F$ that decays by way of the bacteriochlorophyll triplet state $P^R$. At room temperature, a 2-kG field decreases the quantum yield of $P^R$ by about 40 %. In carotenoid-containing reaction centers, the yield of the carotenoid triplet state which forms via $P^R$ is reduced similarly. The effect of the field depends monotonically on field-strength, saturating at about 1 kG. The effect decreases at lower temperatures, when the yield of $P^R$ is higher. Magnetic fields do not significantly affect the formation of the triplet state of bacteriochlorophyll in vitro, the photooxidation of *P*-870 in reaction centers at moderate redox potential, or the decay kinetics of states $P^F$ and $P^R$.

The effects of magnetic fields support the view that state $P^F$ is a radical pair which is born in a singlet state but undergoes a rapid transformation into a mixture of singlet and triplet states. A simple kinetic model can account for the effects of the field and relate them to the temperature dependence of the yield of $P^R$.

## INTRODUCTION

Recent experiments indicate that the photooxidation of *P*-870 in the reaction centers of photosynthetic bacteria proceeds via a radical pair intermediate state [1–6]. The radical pair (called state $P^F$) forms in less than 10 ps following flash excitation. It decays with a half-time of about 200 ps, transferring an electron to the Fe · ubiquinone complex (X) which serves as the ultimate electron acceptor. In state $P^F$, the reaction center evidently contains the cationic radical *P*-870$^+$ and an anionic radical ($I^-$) of an intermediate electron carrier which most likely is bacteriopheophytin. The evidence for this comes primarily from the similarity of the $P^F$ absorption spectrum to the sum of the spectra of *P*-870$^+$ and the bacteriopheophytin anion.

If the photochemical electron transfer reaction is blocked by chemical reduction of the quinone, the lifetime of state $P^F$ increases from 200 ps to about 10 ns [7, 8].

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Under these conditions, $P^F$ appears to decay by back reactions along two alternate routes One of these returns P-870 directly to the ground (singlet) state The other proceeds by way of a metastable triplet state of *P*-870, called state $P^R$. If $P^R$ is formed in the presence of a strong magnetic field, its ESR spectrum reveals an unusual spin polarization [6, 9]. As we shall discuss below, the polarization appears to reflect the path by which the triplet state is created.

Most chemical reactions are not significantly influenced by the application of a static magnetic field. This is not surprising, because the splittings in energy levels caused by magnetic fields are usually completely negligible compared to thermal energies. However, magnetic fields can have substantial effects on reactions which proceed through radical pairs [10–17]. The effects arise because a magnetic field can influence the interconversion of radical pairs between their singlet and triplet forms, and singlet and triplet radical pairs will, in general, have different fates. Analysis of the effects of the field can help one to determine the initial spin multiplicity of the radical pair and can provide information on the interaction between the two halves of the radical pair.

In the present work, we have studied the effect of magnetic fields on the conversion of state $P^F$ into the triplet state $P^R$.

## MATERIALS AND METHODS

Reaction centers from *Rhodopseudomonas sphaeroides* strains R-26 and 2.4 1 were isolated essentially as described by Clayton and Wang [18] and Cogdell et al. [8], and suspended in 50 mM Tris · HCl, pH 7.6, 0.05 % Triton X-100 Bacteriochlorophyll was extracted from lyophilized cells of strain R-26 with 2 % methanol in petroleum ether, and partially purified by extraction from the petroleum ether into 95 % methanol/5 % $H_2O$.

Absorbance changes following laser flash excitation were measured essentially as described by Parson et al. [7]. For most experiments, Q-switched ruby laser flashes of about 20 ns duration were used Relative quantum yields were determined by attenuating the flash with neutral density filters until the linear portion of the light saturation curve was reached. Under these conditions the amplitude of the flash-induced absorbance change is a measure of the quantum yield of the species monitored.

An electromagnet was constructed out of 2-cm square iron bar and 12-gauge copper wire. A hole was drilled in one pole piece to allow entrance of the laser light parallel to the field direction. The sample was held in a 1-cm cuvette directly in the magnet gap, and the monochromatic measuring light beam was perpendicular to the field direction. The magnetic field strength was measured with an 824 S Rawson-Lush rotating coil gaussmeter (Rawson Inst. Cambridge, Mass).

For low-temperature experiments, a plexiglass cell with a 1.7-mm pathlength was placed at a 45° angle to both the actinic and measuring paths in a Dewar containing liquid $N_2$. For intermediate temperatures, nitrogen gas cooled by passage through a copper coil immersed in liquid $N_2$ was blown onto the sample. The temperature was controlled by varying the gas flow rate, and was measured by a Cu/constantan thermocouple immersed directly in the sample. Glycerol (60 %) or ethylene glycol (50 %) was included in the samples for the measurements at low temperatures. This change in the solvent had no effect at room temperature.

## RESULTS

Figs. 1A and 1B show the formation of the triplet state $P^R$ upon flash excitation of chemically reduced reaction centers from *Rhodopseudomonas sphaeroides* strain R-26, in the presence of a negligibly small magnetic field and in the presence of a 2-kG field. The strong field decreases the amount of $P^R$ that is formed by about 40 %. It has no effect on the decay kinetics of the triplet state. The field also has no significant effect on the decay kinetics of state $P^F$, the biradical which gives rise to state $P^R$ (Figs. 1C and D)

In bacteria which contain carotenoids, the triplet state of *P*-870 is not observed at room temperature. Instead, one sees a species with an absorption spectrum consistent with a carotenoid triplet state [8]. Studies at low temperatures have shown that the carotenoid triplet forms from state $P^R$ by energy transfer [19]. Figs. 1E and F show the effect of a 2-kG magnetic field on the formation of the carotenoid triplet state in reaction centers from *R. sphaeroides* strain 2.4.1. The field causes a decrease of approximately 50 % in the amount of the triplet that is formed, again with no significant effect on the decay kinetics.

The measurements shown in Figs. 1E and F were made with samples that had been stored at low redox potential for several days at 5 °C. In an extensive series of measurements the ratio of the amount of carotenoid triplet that was formed at high field to that formed at low field ($\Phi_H/\Phi_0$) was 0.51 ±0.05. In another series employing a freshly prepared sample, the effect of the field was smaller, giving an average of 0.74 ± 0.03 for $\Phi_H/\Phi_0$. After ageing for two days at low redox potential, this sample had a $\Phi_H/\Phi_0$ of 0.55±0.06. Fig. 2 collects similar data from a large number of experiments; aged samples have consistently lower values than fresh ones. No change in the

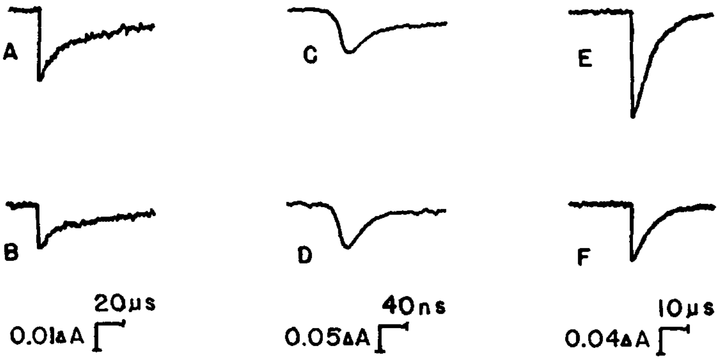

Fig 1. Flash-induced absorbance changes in dithionite-reduced reaction centers of *R sphaeroides*. A downward deflection is an absorbance increase (A) $P^R$ formation monitored at 510 nm in R-26 reaction centers at $H = 25$ G. Incident flash intensity 3.7 neinsteins · $cm^{-2}$ Reaction center concentration 5.7 µM. (B) Same as A except $H = 2000$ G (C) $P^F$ formation monitored at 420 nm in R-26 reaction centers at $H = 25$ G. Reaction center concentration 6.0 µM. (D) Same as A (except $H = 1750$ G). (E) Carotenoid triplet formation monitored at 545 nm in 2.4.1 reaction centers at 25 G Flash intensity 2.3 neinsteins · $cm^{-2}$. Reaction center concentration 6.0 µM (F) Same as E except $H = 2000$ G. The field of 25 G in (A), (C), and (E) was due to residual magnetization of the electromagnet Within experimental error, measurements made in the presence of this field were indistinguishable from those obtained when *H* was reduced to zero by reversing the current in the coil.

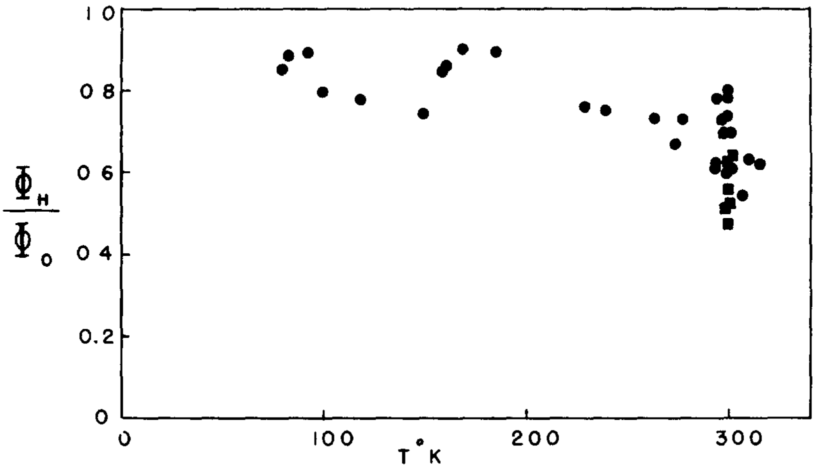

Fig 2 Temperature dependence of the effect of a 1750 G magnetic field on flash-induced carotenoid triplet formation monitored at 545 nm in dithionite-reduced 2.4 1 reaction centers Points near 300 K as in Fig. 1E and F. Lower temperatures as described in Materials and Methods ●, experiments with freshly prepared samples ■, experiments with samples aged at low potential Each point represents the average of from 4 to 12 pairs of measurements.

absorption spectrum occurred on ageing, and the origin of the ageing effect remains obscure. Reaction centers from strain R-26 behaved similarly in this regard, but were not studied as extensively. The effect of the field depended also on the width of the excitation flash, being somewhat more pronounced with 7-ns flashes than with the 20-ns flashes that we used routinely.

The quantum yield of $P^R$ is approximately 0.1–0.2 at room temperature [7]. If one lowers the temperature, the quantum yield slowly increases, approaching 1 at 20 K [7]. Fig. 2 shows the effect of the magnetic field as a function of temperature. The magnitude of the effect decreases gradually as the temperature is lowered. A typical experiment with freshly prepared samples yielded a $\Phi_H/\Phi_0$ of $0.73\pm0.06$ at room temperature and $0.88\pm0.11$ at liquid nitrogen temperature. Measurements at intermediate temperatures are not as accurate, due to the difficulties involved in maintaining a stable temperature long enough to perform a large number of measurements.

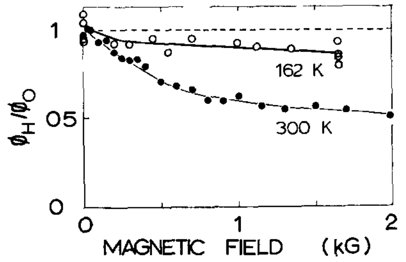

Fig 3. Magnetic field dependence of carotenoid triplet formation in dithionite-reduced 2 4 1 reaction centers ●, 300 K, conditions as described in Fig 1E ○, 162 K, conditions as described in Materials and Methods.

Fig. 3 shows the dependence of the yield of the carotenoid triplet state on the strength of the field at 300 and 162 K. The decrease in triplet yield is monotonic, with the effect saturating at approximately 1 kG at 300 K. The data at 162 K are not accurate enough to determine if the effect saturates at the same field strength.

Because photomultiplier tubes are subject to interference by magnetic fields, it is necessary to consider whether the effects shown in Figs. 1–3 are due to instrumental artifacts. To test this point, we examined the formation of the triplet state of bacteriochlorophyll in vitro. This occurs by conventional intramolecular intersystem crossing rather than by way of a radical pair, and one would not expect it to be influenced by a magnetic field. Figs. 4A and B show that there was indeed no effect.

Figs. 4C and D show the formation of $P$-870$^+$ in reaction centers at moderate redox potential; a 2-kG magnetic field has no effect on the quantum yield of $P$-870$^+$.

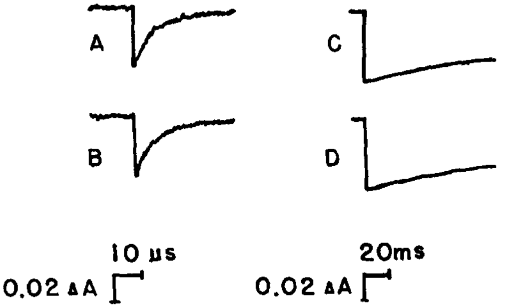

Fig 4. (A) Flash-induced bacteriochlorophyll triplet formation in anaerobic 95 % methanol/5 % water ($A_{770}$ = 1 7) $H$ = 25 G, flash intensity 3 7 neinsteins · cm$^{-2}$ (B) Same as A except $H$ = 1750 G (C) $P$-870$^+$ formation monitored at 430 nm in 5 0 µM 2 4 1 reaction centers at moderate potential $H$ = 25 G, flash intensity 0 48 neinsteins · cm$^{-2}$. (D) same as C except $H$ = 1750 G.

## DISCUSSION

Chloroplasts, algae, and whole cells of some types of photosynthetic bacteria will orient in a magnetic field [20–21]. We feel that the field effects described in the present paper are not due to orientation, because field-induced orientation usually requires large particles and high field strengths [20–21]. The small size of the reaction center complex and the low field strengths required to cause the decrease in triplet yield argue strongly against orientational effects. A more satisfactory explanation can be found by considering the sequence of steps that lead to the formation of state P$^R$.

When $P$-870 is excited by light, the transfer of an electron to I occurs within 10 ps [1–5] Because the electron transfer is so rapid, the radical pair state P$^F$ almost certainly is born in a singlet spin state. But if the two unpaired electrons of the radical pair are sufficiently far apart, the coupling between them will be weak, and the relative orientations of their spins may change before P$^F$ decays. If the return of an electron from I$^-$ to $P$-870$^+$ occurs while P$^F$ is in a singlet state, the result presumably is to restore $P$-870 directly to the ground singlet state. Return of an electron from a triplet state of the radical pair places $P$-870 in the triplet state, P$^R$. The quantum yield of P$^R$, therefore, depends on the probability that the radical pair will be in a triplet state at any given time.

Because of differences in $g$ factor, nuclear hyperfine coupling and spin-spin interactions, the two unpaired electrons of $P^F$ experience different local magnetic fields. The behavior of such systems has been discussed by several authors, mainly in connection with the phenomena of chemically-induced dynamic nuclear and electron spin polarization [22–25] An introduction to magnetic field effects has been given by Atkins [15], and useful theoretical treatments by Atkins and Lambert [16], Brocklehurst [14], and Schulten et al. [17]. In general, a radical pair will oscillate between singlet and triplet states, with the frequency and amplitude of the oscillation depending on the differences between the local magnetic fields and on the coupling between the two electrons. Let us assume for the moment that the oscillation frequency is high relative to the rate at which $P^F$ decays. In the absence of an external field, the detailed time dependence of the system still can be quite complex, because the nuclear and electronic spins which influence the electrons of the radical pair can take on any orientation in space. In the ideal case that hyperfine and exchange interactions are completely isotropic and the dipolar and exchange coupling between the two electrons weak, the three zero-field substates of the radical pair ($T_x$, $T_y$, and $T_z$) will all be nearly degenerate with the singlet state, and any interaction which interconverts singlet and triplet states will form all three triplet states with equal probability. The average probability that the radical pair is in a triplet state then will be approximately three times the average probability that is in the singlet state.

Placing the system in an external magnetic field simplifies the situation by aligning all of the nuclear and electronic spins with respect to the direction of the field.

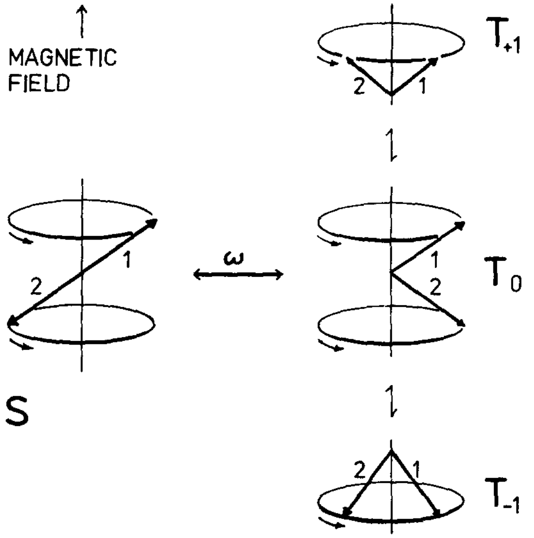

Fig 5 Vector diagram illustrating singlet-triplet interconversion of a radical pair at high magnetic field. Arrows labeled 1 and 2 represent spin angular momentum vectors for the unpaired electrons on radicals 1 and 2. These vectors precess counterclockwise about the field axis, $z$ In the singlet state the two electrons are arranged antisymmetrically so that the magnitude of the resultant vector is zero In the three triplet levels the resultant vector has magnitude $\sqrt{2}\hbar$ with $z$ components of $\hbar$, 0, and $-\hbar$ for the $T_{+1}$, $T_0$, and $T_{-1}$ states respectively Interconversion of S and $T_0$ states occurs if the precession frequencies of spins 1 and 2 are not equal.

Under these conditions, the radical pair oscillates between the singlet state and only one triplet state, $T_0$. These states have no net spin magnetic moment in the direction of the field, and they continue to be nearly degenerate. Fig 5 shows schematically how the oscillation between them occurs The $T_{+1}$ and $T_{-1}$ states, which are split apart in energy by the field, do not equilibrate rapidly with the singlet state, although they can be populated from S and $T_0$ by spin-lattice relaxation.

The unusual electron spin polarization which $P^R$ exhibits supports the idea that an external field prevents $P^F$ from entering the $T_{\pm 1}$ triplet sublevels. One would expect the $T_0$ polarization of $P^F$ to be retained in the back-reaction which generates $P^R$, and this evidently is the case. In high fields, and at temperatures below 10 K (when spin-lattice relaxation is very slow), $P^R$ is created almost exclusively in the $T_0$ sublevel [6, 9] Schaafsma et al. [26] and Thurnauer et al. [6] have pointed out that the electron spin polarization that is observed in $P^R$ is inconsistent with intramolecular intersystem crossing, but can be explained by a radical pair mechanism. Brocklehurst [12] also has shown that triplets formed from a radical pair would have $T_0$ polarization.

Still assuming that the oscillation between the singlet and triplet state is fast compared to the decay of $P^F$, and assuming further that spin-lattice relaxation is slow, the quantum yield of $P^R$ will be

$$\Phi = k_T f/[k_T f + k_S(1-f)], \tag{1}$$

where $k_S$ and $k_T$ are rate constants for the electron transfer from $I^-$ to $P$-$870^+$ in the singlet and triplet states of $P^F$, and $f$ is the fraction of $P^F$ that is in a triplet state. Taking the value of $f$ as $\frac{3}{4}$ in zero field and $\frac{1}{4}$ in high field, the ratio of the yields of $P^R$ in the presence and absence of the field would be

$$\frac{\Phi_H}{\Phi_0} = \frac{1 + k_S/3k_T}{1 + k_S/k_T} \tag{2}$$

Eqn. 2 predicts a field effect of $\Phi_H/\Phi_0 = 0.33$ when $k_S/k_T \gg 1$. The effect will be smaller than this if spin-lattice relaxation allows the value of $f$ to approach $\frac{3}{4}$ even in the presence of the field, and it will be smaller if $k_S$ is not much greater than $k_T$. Fig. 6 shows how the effect of the field is related to the quantum yield of $P^R$. The effect will disappear as $\Phi_0$ approaches 1.0, i.e. when $k_S/k_T \ll 1$. This accounts for the decrease of the effect at low temperatures (Fig. 2), if the increase in $\Phi_0$ with decreasing temperature reflects a change in the ratio of the rate constants. The disappearance of the effect of the field at low temperatures agrees with previous evidence that the quantum yield of $P^R$ approaches 1 at low temperature, both in the absence [7] and presence [27] of a field.

The observed effect of the field is smaller than the maximum possible effect, both at room temperature and at 80 K (Fig. 6). This could be due to spin-lattice relaxation of $P^F$. The 10-ns lifetime is short enough so that one might not expect spin-lattice relaxation to occur, but the presence of Fe in the reaction center complex makes estimation of the relaxation time uncertain. If spin-lattice relaxation does occur during the lifetime of $P^F$, it would complicate the temperature dependence of the magnetic field effect. As one lowered the temperature, a decrease in the spin-lattice relaxation rate could partially offset the influence of the increasing value of $\Phi_0$

In principle, the magnetic field should cause a slight decrease in the rate of

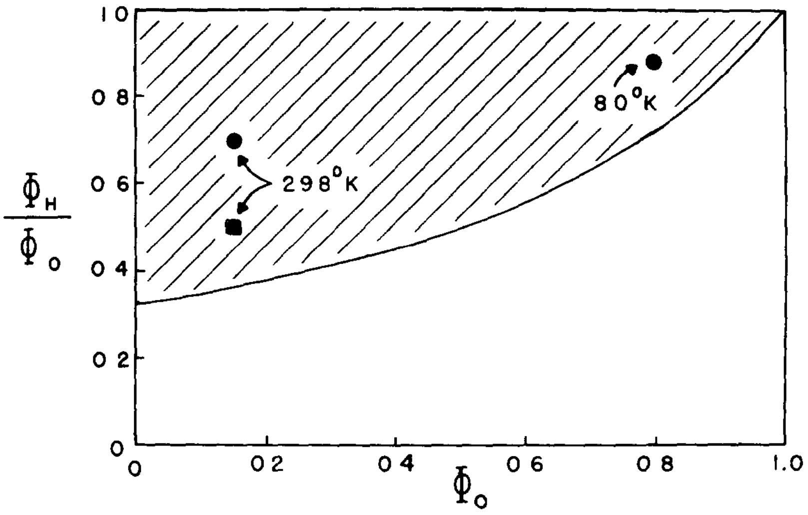

Fig 6 Theoretical plot of field effect as a function of zero-field triplet quantum yield This diagram is a plot of the equation $\Phi_H/\Phi_0 = 1/(3-2\Phi_0)$, readily obtained using Eqn 1 The line is the maximum effect possible in the absence of spin-lattice relaxation. The shaded area represents all possible values $\Phi_H/\Phi_0$ may take on depending on the extent of spin-lattice relaxation of $P^F$ This plot assumes that $P^F$ is born as a singlet radical pair and that singlet-triplet interconversion is fast relative to the $P^F$ decay rate The data points represent typical $\Phi_H/\Phi_0$ values for samples at 298 and 80 K The values of $\Phi_0$ were estimated from Fig 4 of ref 7 ●, freshly reduced samples, ■, aged samples

decay of state $P^F$. However, the predicted change is only about 10 %, and is beyond the accuracy of the measurements shown in Figs. 1C and D.

We have assumed above that the oscillation between the singlet and triplet forms of $P^F$ is rapid, relative to the rate of decay of $P^F$. This assumption seems reasonable, because the yield of $P^R$ can be close to 1 0, at least at low temperatures. If the oscillation frequency were low relative to $k_S$, $P^F$ would decay to the ground state before it developed any triplet character, limiting the quantum yield of $P^R$. A more complete discussion, considering the factors that influence the oscillation frequency and considering the possibility of a temperature-dependent frequency, will be presented elsewhere.

Finally, the lack of a field effect of $P$-870$^+$ formation merits some discussion. Since $P$-870$^+$ $X^-$ formation is very rapid (200 ps) in reaction centers at moderate potential, it is probable that electron transfer to form $X^-$ occurs before singlet-triplet rephasing in $P^F$ can occur. However, the difference in energy between the singlet and triplet forms of $P^F$ is undoubtedly small compared to the energy gap between $P^F$ and $P$-870$^+$ $X^-$ It is therefore unlikely that the spin state of $P^F$ would have any significant influence on the rate of the electron transfer from $I^-$ to X.

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