

The relation between the electron spin polarization of the donor triplet state of the photosynthetic reaction center from *Rhodopseudomonas viridis* and the redox state of the primary acceptor

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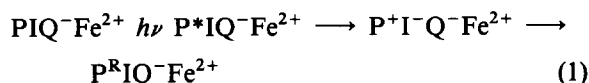
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The hypothesis [(1986) Photobiochem. Photobiophys. 11, 95–100] that the temperature dependence of the electron spin polarization (ESP) pattern of the $\Delta m = \pm 1$ EPR spectrum of the triplet state P^R of the *Rhodopseudomonas viridis* reaction center is caused by magnetic interaction between the reduced menaquinone-iron complex Q^-Fe^{2+} and the electron spin on I^- (reduced bacteriopheophytin b), which is part of the radical pair P^+I^- (P^+ is the oxidized primary electron donor P960) has been investigated. It was found that the AEAEAE ESP pattern of the EPR spectrum detected at $T > 20$ K changes into the usual AEEAAE pattern, when Q^-Fe^{2+} is photochemically converted into Q^2-Fe^{2+} . This demonstrates that the presence of Q^- in Q^-Fe^{2+} is a necessary condition to obtain the AEAEAE ESP pattern.

Triplet state electron spin polarization Bacterial photosynthesis EPR Reaction center Redox state

1. INTRODUCTION

In bacterial photosynthetic reaction centers (RCs) the donor triplet state P^R is generated under illumination when the primary acceptor, a quinone-iron complex (QFe^{2+}), has been reduced [1–3]:



where P is the primary electron donor ($BChl_2$) and I is the intermediary acceptor ($BPheo$). At low temperature the radical pair P^+I^- (P^F) recombines exclusively forming P^R [3,4]. In high external magnetic fields this occurs via the radical pair mechanism [5,6], resulting in a triplet state with an

electron spin polarization (ESP) pattern that has been shown to be inconsistent with an intramolecular intersystem crossing (ISC) mechanism [7,8], and characteristic for photochemical activity in photosynthetic RCs. This triplet state has been detected by means of EPR spectroscopy in numerous photosynthetic species, including plant photosystem I and II, and green bacteria [1,9–12], invariably showing an AEEAAE ESP pattern (A, enhanced absorption; E, emission). However, we observed deviating ESP patterns in the $\Delta m = \pm 1$ EPR triplet spectra of P^R in isolated RCs from the purple bacterium *Rhodopseudomonas viridis* (AEAEAE) and in chromatophores of *Chromatium vinosum* (AE–EA) at temperatures above 20–25 K [13,14]. Spin-lattice relaxation within P^R or intramolecular ISC are unlikely to cause this change in the polarization pattern. The phenomenon was ascribed to a magnetic interaction between the electron spin on I^- , which is a part of the radical pair P^+I^- , and a third electron

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spin on the reduced menaquinone Q^- in the Q^-Fe^{2+} complex. This complex transmits the rapid relaxation of the high-spin Fe^{2+} to I^- .

Although SDS treatment is known to uncouple at least the iron atom from the QFe^{2+} complex [15], this method is not so well-defined as the technique of iron removal in RCs from *Rps. sphaeroides* R26 [16,17]. Therefore, a stronger proof for the involvement of Fe^{2+} and/or Q in the polarization inversion of the Y peaks of P^R in this system is desirable. Unfortunately, the iron-removal technique using $LiClO_4$ and *o*-phenanthroline does not apply to RCs from *Rps. viridis*, nor can Q be removed easily. We therefore have investigated the triplet polarization pattern as a function of the redox state of the primary acceptor. Since the *Rps. viridis* RC contains a fast donating cytochrome, it is possible to reduce doubly the quinone acceptor. This is expected to quench the magnetic interaction between the quinone-iron complex and the radical pair P^+I^- .

2. EXPERIMENTAL

RCs were isolated according to [14] and concentrated to $A_{830} = 50 \text{ cm}^{-1}$. EPR experiments were carried out as described in [14]; a typical EPR sample contained 25 μl of 0.5 M sodium ascorbate, 75 μl RCs, and 200 μl ethylene glycol. To create the doubly reduced state $Q^{2-}Fe^{2+}$, the samples were illuminated at room temperature with white light ($\sim 0.5 \text{ W} \cdot \text{cm}^{-2}$) from a 150 W xenon lamp (Eimac R150-7A) filtered through 6 cm water for varying periods of time, followed by rapid cooling to 77 K. Exposure to UV light was avoided. Reoxidation of $Q^{2-}Fe^{2+}$ was accomplished by flushing the sample with oxygen for several minutes at room temperature [18]. 2 μl ascorbate was added to these samples, which were then refrozen in the dark. Q_A to Q_B electron transfer was blocked by adding $\sim 5 \text{ mM}$ *o*-phenanthroline to the EPR samples.

3. RESULTS

The treatment of the RCs, in order to produce the $Q^{2-}Fe^{2+}$ state, is very similar to that on *Rps. sphaeroides* R26 RCs by Okamura et al. [18], and on *Rps. viridis* RCs by Prince et al. [19]. Fig. 1 presents the Q^-Fe^{2+} , I^- and light-induced triplet

EPR spectra, when the RCs had been exposed to white light at room temperature for different periods of time. Prince et al. [19] found that upon illumination under these conditions the triplet spectrum completely disappears, due to trapping of I^- , inhibiting any further charge separation. To be able to observe P^R we had to use a moderate redox potential, allowing Q^{2-} production, and simultaneously permitting reoxidation of I^- . We used ascorbate at pH 8 ($E^{\circ'} = 0 \text{ mV}$), so the Q^-Fe^{2+} state had to be generated by illumination, since the Q/Q^- redox couple is $\sim -150 \text{ mV}$ [20].

Upon increasing the period of illumination, the EPR signal at $g = 1.8$ of Q^-Fe^{2+} [21,22] disappears. Concurrently, the spin polarization of the Y^-/Y^+ peaks in the triplet spectrum changes from A/E into E/A (fig. 1b,c), whereas at low microwave power a $g = 2$ signal appears, due to trapping of I^- [19,23]. The $I^-Q^-Fe^{2+}$ spectra can be understood by considering the results of the experiments represented in fig. 1a–c: although the sample was not illuminated at 300 K (fig. 1a) a split signal (S in fig. 1a) is observed, due to the trapping of a small amount of I^- , competitively formed during the generation of Q^- by illumination at 10–20 K. (When the sample is left in the dark, no split signal is observed.) After 20 s of illumination at 300 K (fig. 1b), Q^{2-} is built up at the cost of Q^- and more I^- has been trapped, as is evident from fig. 1 (center). After 120 s of illumination at 300 K (fig. 1c), the sample contains four different photoproducts: (i) IQ^- , (ii) I^-Q^- , (iii) IQ^{2-} , (iv) I^-Q^{2-} . From the amplitude of the $g = 1.8$ signal an estimated fraction of less than 5% of these photoproducts were in states i and ii. Of the remaining 95%, state iii gives rise to the observed P^R triplet spectrum, whereas state iv is detected as I^- , but does not give rise to triplet formation. Formation of state iv explains the reduced amplitude of the EPR triplet spectrum. Prolonged illumination results in a lower triplet yield, due to the increased fraction of I^-Q^{2-} . The EPR triplet spectrum of fig. 1c does not show any changes in the 8–100 K temperature range, except for some decrease in amplitude of all peaks.

When the sample of fig. 1c is thawed, flushed with oxygen and refrozen in the dark, the Q^-Fe^{2+} signal reappears, and the ESP pattern of P^R at 110 K is converted into the original AEAEAE pattern. When the experiments are repeated with

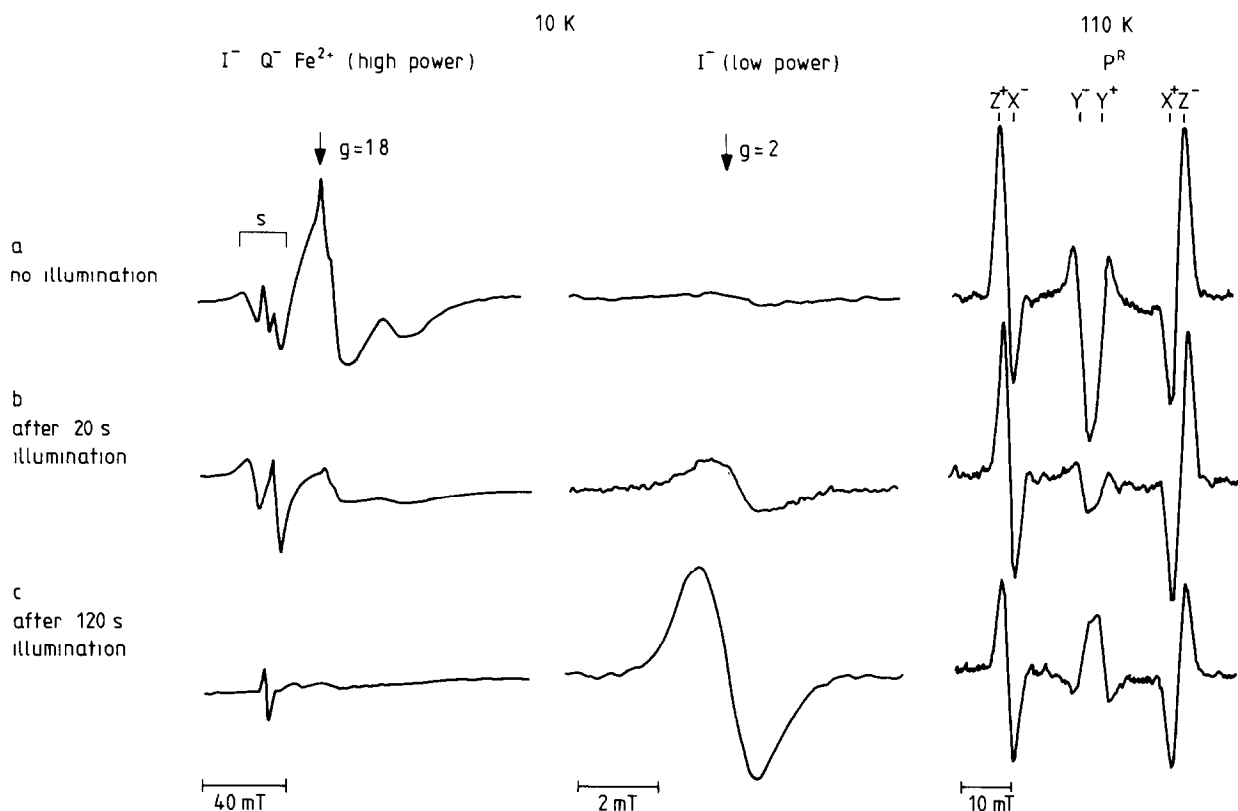


Fig.1. Relation between electron spin polarization pattern of the EPR triplet spectrum of P^R in isolated RCs of *Rps. viridis* and the redox state of the primary acceptor. All samples contained 4 mM sodium ascorbate ($E^{\circ'} = 0$ mV) pH 8, 200 μ l ethylene glycol, 75 μ l RCs ($A_{830} = 50$ cm $^{-1}$) and were photochemically reduced. (a) No illumination at 300 K (only low-temperature illumination to produce Q^-), P^R spin polarization pattern: AEAEAE, s = split signal (I^-Q^-); (b) intermediate case, after 20 s of 300 K illumination; (c) after 120 s illumination, P^R polarization pattern: AEEAAE. Instrumental settings: $I^-Q^-Fe^{2+}$ spectra: 3.2 mT modulation amplitude, 5 mW, I^- (low-power) spectra: 0.4 mT modulation, 2 μ W, P^R spectra: 1 kHz light modulation, 2.5 mT field modulation, 5 mW. All spectra have the same vertical scale.

samples in which the electron transfer from Q_A to Q_B is blocked, identical results are obtained.

4. DISCUSSION

In *Rps. viridis* RCs the magnetic I^-Q^- interaction is relatively large (~ 15 – 20 mT [19]) as compared to other bacteria (e.g. *Rps. sphaeroides* ~ 0.1 – 0.5 mT [18,24], *Chromatium vinosum* ~ 6 mT [23]). Therefore, we ascribed the observed effects to a spin-spin interaction between Q^-Fe^{2+} and P^+I^- [14].

In the left wing of the Q^-Fe^{2+} spectrum in fig.1 the I^- high-power spectrum can be recognized.

This split I^-Q^- signal (S in fig.1) disappears concomitantly with the Q^-Fe^{2+} signal and the A/E polarization of the Y^-/Y^+ peaks, resulting in the AEEAAE ESP pattern, characteristic for the radical pair mechanism. It can be concluded that the presence of the paramagnetic species Q^-Fe^{2+} is essential to observe the AEAEAE polarized P^R triplet spectrum at $T > 20$ K. There are several possible explanations: (i) Direct magnetic (dipolar) coupling between Q^-Fe^{2+} and P^R as suggested to exist in *Rps. sphaeroides* [25]. This is unlikely, since it cannot explain the relation [14] between I^-Q^- coupling strength and the observed P^R ESP pattern of the triplet spectrum in *Rps. viridis*, *C.*

vinosum and *Rps. sphaeroides*. For the latter we did not observe a temperature-dependent change in polarization pattern. (ii) The presence of Q^{2-} converts the high-spin Fe^{2+} into its low-spin form and subsequently changes the interaction with P^+I^- . This indeed may explain the experimental results, if Fe^{2+} is the source of the temperature effect on the spectra of P^R . Although there is no firm proof that the spin state of Fe^{2+} does not change when the primary quinone is doubly reduced, addition of *o*-phenanthroline to the RCs, blocking the electron transfer step between Q_A and Q_B [26], does not affect our experimental results. Butler et al. [27] have observed the characteristic $g = 1.83$ EPR signal in the $Q_B^-Fe^{2+}Q_A^-$ state. Under our experimental conditions the Fe^{2+} is ligated to Q_A^- and Q_B , which together are expected to have a smaller effect on the iron spin state. Furthermore, susceptibility measurements on *Rps. sphaeroides* R26 RCs did not show any changes in the spin state of the iron on reduction of the primary acceptor [16]. It is therefore unlikely that the Fe^{2+} spin state in *Rps. viridis* RCs changes upon illumination, due to the reduction of the quinone(s). (iii) Q^- acts as a carrier of spin transitions between the high-spin Q^-Fe^{2+} and P^+I^- . The $P^+I^-Q^-Fe^{2+}$ must be considered as a multispin system, to which the conventional radical pair mechanism cannot be applied as is reflected by the observed temperature-dependent change of the P^R triplet pattern. Doubly reducing the primary quinone restores the radical pair mechanism as demonstrated by the AEEAAE polarization pattern.

The $Fe^{2+}Q^-$ complex is coupled to the P^+I^- radical pair state and causes the ESP pattern of P^R to change at higher temperatures. Consequently, monitoring the EPR triplet state not only yields information about the photochemical activity of the P-I part in the RC (i.e. AEEAAE below 15–20 K). Well above this temperature the observed ESP pattern also demonstrates that the Q_AFe^{2+} part is active in the RC. This provides a tool to check the acceptor side in RC or chromatophore preparations from *C. vinosum* and *Rps. viridis* with EPR at liquid nitrogen temperatures.

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