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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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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]:

$$PIQ^-Fe^{2+} \; h\nu \; P^*IQ^-Fe^{2+} \longrightarrow P^+I^-Q^-Fe^{2+} \longrightarrow P^RIQ^-Fe^{2+} \quad (1)$$

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 $A_{830} = 50\ cm^{-1}$. 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 (~0.5 $W \cdot cm^{-2}$) 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 ~5 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 ($E^{o\prime}$ = 0 mV), so the Q^-Fe^{2+} state had to be generated by illumination, since the Q/Q^- redox couple is ~ −150 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

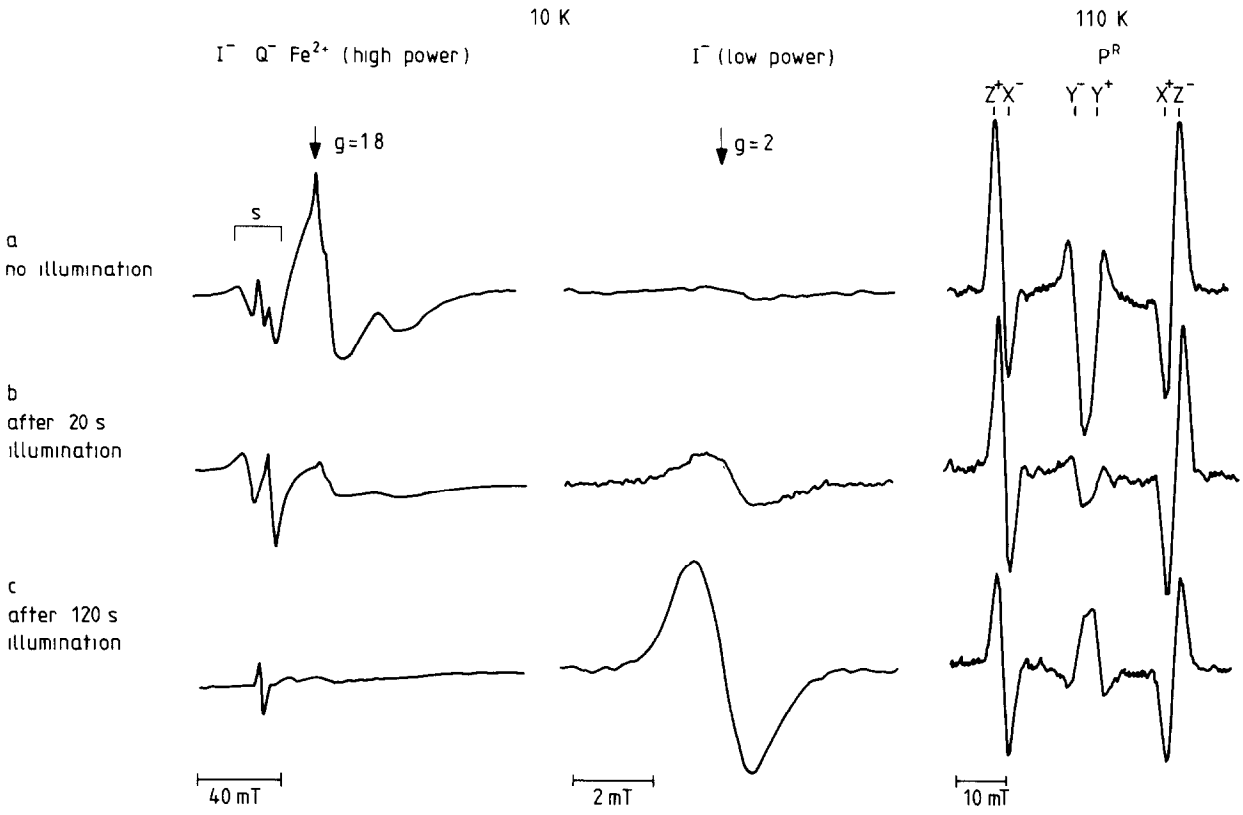

Fig.1. Relation between electron spin polarization pattern of the EPR triplet spectrum of P^R in isolated RCs of *Rps. viridis* and the redox state of the primary acceptor. All samples contained 4 mM sodium ascorbate ($E^{o\prime}$ = 0 mV) pH 8, 200 μl ethylene glycol, 75 μl RCs (A_{830} = 50 cm^{-1}) 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 μW, 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 $Q_B^{2-}Fe^{2+}Q_A^-$ state. Under our experimental conditions the Fe^{2+} is ligated to Q_A^{2-} 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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