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# Formation of charge separated state $P^+Q_A^-$ and triplet state $^3P$ at low temperature in *Rhodobacter sphaeroides* (R-26) reaction centers in which bacteriopheophytin *a* is replaced by plant pheophytin *a*

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**Abstract** Low temperature optical and photochemical properties of *Rhodobacter sphaeroides* (R-26) reaction centers, in which bacteriopheophytin *a* has been replaced by plant pheophytin *a*, are reported. Modified reaction centers preserve the ability for photoinduced electron transfer from the primary electron donor P to the primary quinone acceptor $Q_A$ at 80K. The triplet state ESR signal of modified reaction centers with prereduced $Q_A$ at 10K shows an electron spin polarization pattern and ZFS parameters analogous to those for the triplet state $^3P$ in non-treated reaction centers. It was found that at low temperature both $P^+Q_A^-$ and $^3P$ states are formed via a precursor radical pair $P^+I^-$ in which I is the introduced plant pheophytin molecule. This shows that acceptor systems of bacterial and plant (photosystem II) reaction centers are mutually replacable in structural and functional aspects.

*Key words:* Reaction center; Electron transfer; Pheophytin; Bacteriopheophytin; *Rhodobacter sphaeroides*

## 1. Introduction

Bacterial reaction centers are specialized pigment–protein complexes where the primary electron transfer reaction of photosynthesis takes place. This reaction is a multi-stage process [1] occurring between bacteriochlorophyll-type pigment molecules, the first stable (or primary) acceptor being a quinone molecule. *Rhodobacter sphaeroides* RC structure and composition are well known [2,3]. The RCs comprise 4 BChl molecules, 2 BPhe molecules, 2 ubiquinones and 1 $Fe^{2+}$ ion, embedded in a polypeptide matrix. Their structure–function relationship is less understood. A promising approach to clarify this relationship is an introduction of controlled changes to the structure and the observation of consequent alterations of the RC properties. Recently a new procedure has been worked out [4–6], employing the chemical exchange of endogeneous pigments with those in solution. In our previous work [7,8] we described room-temperature spectroscopic and photochemical properties of *Rb. sphaeroides* R-26 RCs chemically exchanged with pheophytin *a* at the bacteriopheophytin *a* sites $H_A$ and $H_B$ [6]. Here we report on the ability of similar preparations for electron transfer at low temperatures. Also, we report on the observation and characterization of the light-induced triplet state by flash-ESR.

## 2. Materials and methods

Reaction centers were isolated from *Rb. sphaeroides* R-26 chromatophores as described in [9]. Three types of preparations were used in this work: non-treated RCs, modified and control ones. RC modification was performed according to [6]. Briefly, methanolic Phe solution was added to a 8 $\mu$M RC solution to achieve a 20-fold excess of the pigment (relative to the RC concentration) with a final methanol concentration of 10%. The mixture was incubated for 90 min at 42°C in the dark, and solubilized pigment was then removed by chromatography on DEAE cellulose. The control preparation was subjected to the same treatment, but in the absence of Phe. All measurements were performed in a 10 mM Tris-HCl (pH 8.0)/0.1% LDAO buffer containing 60% glycerol. Low-temperature optical spectra were measured in a home-built cryostat at 80K. Light-minus-dark difference absorption spectra were measured point by point with a phosphoroscopic setup [10] at non-saturating actinic light intensity. Circular dichroism spectra were measured as in [9]. Flash-ESR triplet state measurements were done as described earlier [11]. To reduce the primary acceptor ($Q_A$), 10 mM of sodium ascorbate was added to the RCs, which were then cooled to 77K in the light.

## 3. Results

Absorption spectra of modified and non-treated RCs measured at 80K are given in Fig. 1a. The modification procedure leads to the following apparent spectral changes: (i) to a considerable decrease of the 759 nm absorption band belonging to BPhe [12], (ii) appearance of new bands at 674 nm and 510 nm, and (iii) disappearance of the 535 nm BPhe band with a preservation of the 545 nm band. Relative positions and intensities of the persisting bands also slightly change.

In the sercond-derivative presentation of the 80K absorption spectrum of modified RCs a peak at 812 nm appears (data not shown). This peak corresponds to a shoulder on the long-wavelength side of the BChl $Q_Y$ absorption band at 800 nm described earlier for RCs from *Rb. sphaeroides* R-26 [13].

Fig. 2 compares circular dichroism spectra of non-treated and modified RCs at 80K. In accordance with room-temperature measurements [6], modification leads to the disappearance of the BPhe negative CD band at 748 accompanied by a decrease of the amplitude of the positive band at 795 nm. A new positive feature shows up at 674 nm.

Photochemical activity of modified RCs is demonstrated in Fig. 1b. Again, there is a prominent decrease of light-induced absorption changes around the BPhe band at 760 nm as compared to non-treated RCs [9]. A new feature centered at 674 nm

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*Abbreviations:* Bchl, bacteriochlorophyll *a*; (B)Phe, (bacterio)-pheophytin *a*; P, primary electron donor, Bchl *a* dimer; $^3P$, triplet state of P; ZFS, zero field splitting; $Q_A$, primary quinone acceptor; RC, reaction center; cw, continuous wave.


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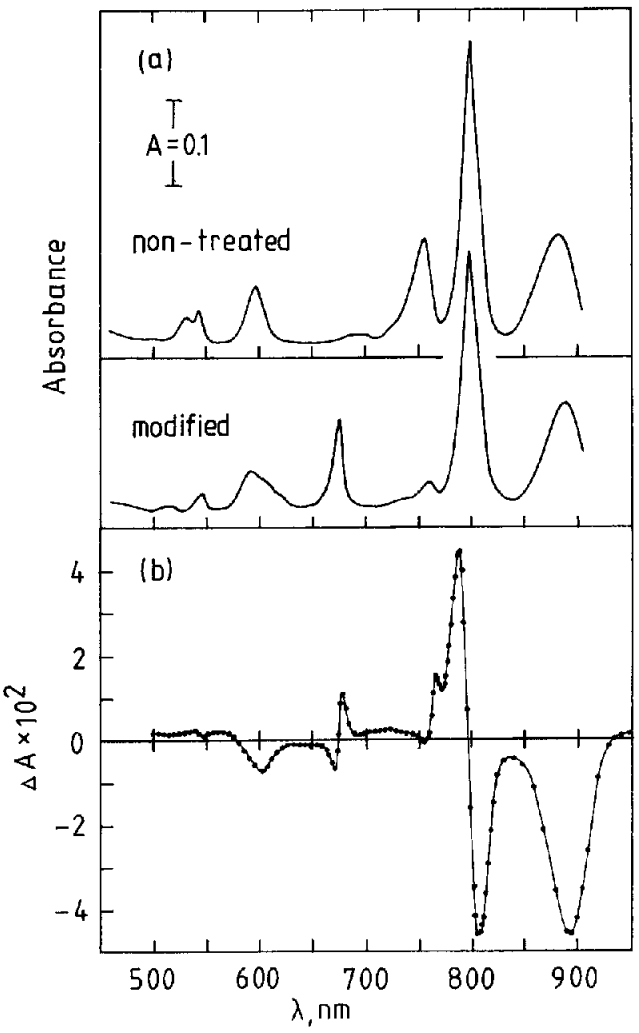


Fig. 1. (a) Optical absorption spectra of non-treated and pheophytin *a*-modified reaction centers from *Rhodobacter sphaeroides* R-26, measured at 80K. (b) Spectrum of reversible absorbance changes ($\Delta A$) induced in pheophytin *a*-modified RCs by actinic light ($\lambda > 640$ nm) at 80 K; $A_{890} = 0.14$.

appears, which may be ascribed to a light-induced long-wavelength shift of the absorption band at 674 nm.

The cw ESR measurements (data not shown) carried out at 70K on non-treated and control RCs demosntrate a typical light-induced signal of $P^+$ [14,15] at $g = 2.0026$ with $\Delta B = 0.98$ mT. In modified RCs a slightly asymmetric light-induced signal at $g = 2.0029 \pm 0.0002$ with $\Delta B = 1.06 \pm 0.02$ mT is detected. In samples of modified RCs reduced with sodium ascorbate a signal at $g = 2.0046 \pm 0.0002$ with $\Delta B = 0.90 \pm 0.02$ mT is detected in the dark.

Triplet-state spectra of reduced modified and non-treated RCs, measured at 10–30K with the flash-ESR technique, are compared in Fig. 3. These spectra exhibit the same electron spin polarization pattern AEEAAE (where A and E refer to microwave absorption and emission, respectively). The values of the ZFS parameters, calculated from the spectra in Fig. 3, are identical for both samples within the experimental error:

$|D| = 0.0188 \pm 0.0001$ cm$^{-1}$
$|E| = 0.0032 \pm 0.0001$ cm$^{-1}$

The values of $|D|$ and $|E|$ do not vary with temperature in the 10–30K range. The ESR signals measured in modified reaction centers are weaker (by a factor of approx. 1.7 at 30K) than those in non-treated RCs under similar experimental conditions.

## 4. Discussion

Optical absorption spectra (Fig. 1a) strongly suggest that the procedure of chemical exchange described in [6] leads to a (partial) removal of BPhe molecules from the *Rb. sphaeroides* R-26 RCs. The new bands detected in modified RCs at 674 nm, 510 nm and 545 nm are close to the bands of Phe in solution [16]; we thus conclude that in accordance with [6] this pigment is incorporated into the RC structure.

A rough estimation, based on the decrease of the 759 nm absorption band (Fig. 1a), shows that in modified preparations approx. 15% of BPhe molecules remain in the RC structure.

The following changes are observed in the CD spectrum of modified RCs, that could be expected to result from pigment exchange (Fig. 2): the modification induces a decrease of the negative band of BPhe at 748 nm and development of a new positive band in the 674 nm region, which is characteristic of the $Q_Y$ transition of Phe. These changes are accompanied by a decrease of the positive 795 nm CD band attributed to the monomeric BChl. Such a decrease may be caused by the loss of excitonic coupling between the monomeric BChl and BPhe, postulated for non-treated RCs [17,18].

Modified RCs preserve the ability for photoinduced electron transfer from P to the primary electron acceptor. Judging from their light-minus-dark spectrum (Fig. 1b), the relatively stable state that gives rise to the observed optical changes is the $P^+$ state [15] with an electron localized most likely on $Q_A$. Optical changes around 760 nm in non-treated RCs are usually associated with electric field-induced long-wavelength shift of BPhe absorption band [10,19]. Their decrease in modified reaction centers, as compared with non-treated RCs [9], is clearly related to partial removal of this pigment. Instead, similar optical changes are observed in the region where the introduced

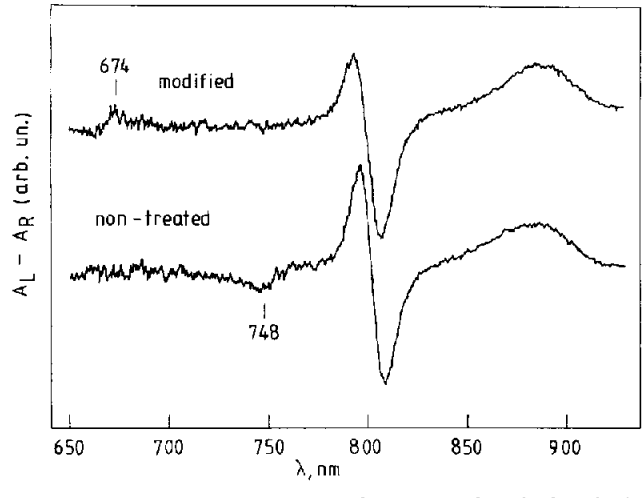


Fig. 2. Circular dichroism spectra of non-treated and pheophytin *a*-modified reaction centers from *Rb. sphaeroides* R-26 at 80K. Spectra were measured with the same preparation as in Fig. 1a.

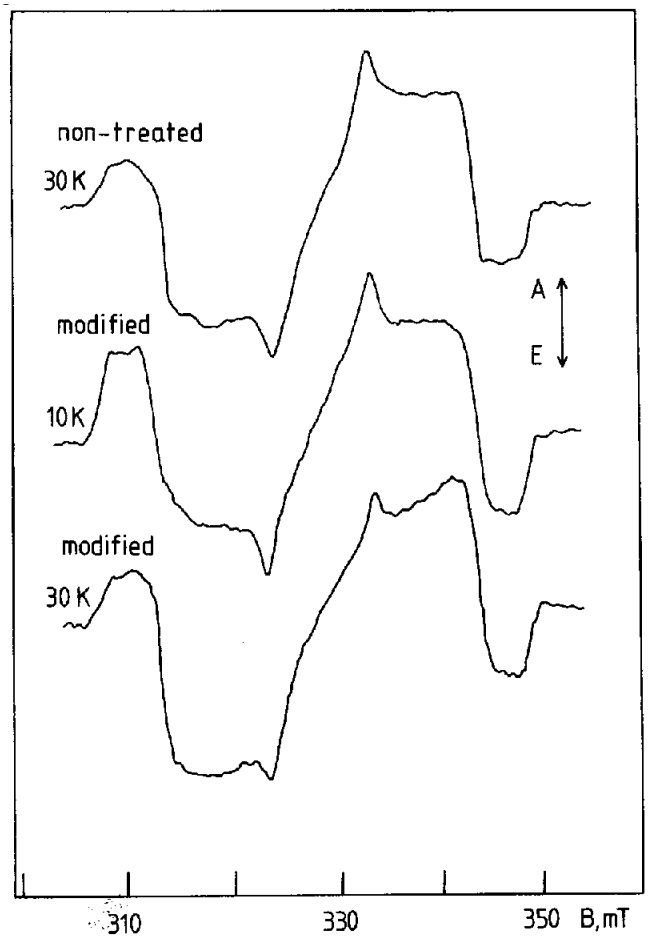


Fig. 3. Flash-ESR triplet state spectra of non-treated and pheophytin *a*-modified reaction centers from *Rb. sphaeroides* R-26 with prereduced primary quinone acceptor $Q_A$. Spectra were normalized to the same peak-to-peak intensity. At 30 K modified RCs give the signal intensity ca. 0.6 of the non-treated ones. Arrows indicate signal signs corresponding to absorption (*A*) and emission (*E*) of microwave power. Excitation at 532 nm (~ 15 ns).

Phe molecules absorb. It looks probable that Phe molecules are subjected to similar electric fields as the BPhe molecules they replace, and are thus incorporated in the RC structure in a similar way.

The cw ESR parameters of modified RCs are at variance with the non-treated RCs. The main reason for this may be due to the disruption of magnetic coupling between $Q_A^-$ and $Fe^{2+}$ ion in a portion of RCs during modification procedure. Computer modelling has shown that the best fit of experimental light-induced spectrum is achieved if approx. 25% of RCs are assumed to lose the coupling. Under reducing conditions this fraction is expected to demonstrate a free-radical signal of the primary acceptor. However, the dark ESR signal of reduced modified RCs has the linewidth slightly larger than that of $Q_A^-$. A superposition of an unidentified additional signal may be suspected.

Flash-induced triplet state ESR signal of modified preparations (Fig. 3) at 10–30K shows electron spin polarization pattern and ZFS parameters analogous to unperturbed $^3P$ [11,20]. The amplitude of the signal at 30K reaches 60% of that in non-treated RCs, whereas only 15% of RCs remain unchanged after the modification procedure. This suggests that modified RCs are capable of generating the triplet state signal with the polarization and ZFS parameters close to those in non-treated RCs. Since the ZFS parameters are sensitive to interaction between the two BChl molecules of the dimeric primary donor P, a conclusion seems feasible that the structure of P is not significantly altered by the modification procedure.

There is general agreement that the AEEAAE polarization of the primary donor triplet state $^3P$ is indicative of a radical pair $P^+I^-$ as the precursor of the $^3P$ state [20]. In non-treated RCs the intermediate acceptor I was shown to be a BPhe molecule (for a review see [15]). The invariance of the AEEAAE pattern in modified RCs implies the absence of drastic changes in the magnetic interactions and sequence of events involved in the formation of $^3P$. Since the modification procedure leads to the replacement of BPhe by Phe, at low temperature the primary radical pair in modified RCs most likely consists of $P^+$ and $Phe^-$ ion-radicals.

The observed decrease of the triplet state ESR signal in modified reaction centers with respect to non-treated RCs (Fig. 3) is, probably, due to a drop in the $^3P$ yield [8,21]. Such drop is expected if the decrease of the lifetime of the $P^+I^-$ state in modified RCs, observed at room temperature [7,8], takes place at low temperature as well.

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