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Biochimica et Biophysica Acta 1321 (1997) 1–9

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

# Reaction centers of *Rhodobacter sphaeroides* R-26 with selective replacement of bacteriopheophytin *a* by pheophytin *a*
# II. Temperature dependence of the quantum yield of $P^+Q_A^-$ and $^3P$ formation

Eric M. Franken [a], Anatoli Ya. Shkuropatov [b], Christof Francke [a], Sieglinde Neerken [a], Peter Gast [a], Vladimir A. Shuvalov [b], Arnold J. Hoff [a], Thijs J. Aartsma [a,*]

[a] *Department of Biophysics, Leiden University, P.O. Box 9504, 2300 RA Leiden, The Netherlands*
[b] *Institute of Soil Science and Photosynthesis, Russian Academy of Sciences, Pushchino, Moscow region 142292, Russian Federation*

Received 22 January 1997; revised 15 April 1997; accepted 17 April 1997

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## Abstract

The quantum yield of the formation of the charge-separated state $P^+Q_A^-$ in reaction centers (RCs) of *Rhodobacter sphaeroides* R-26, in which the bacteriopheophytins in both the active (A) and the inactive (B) branch are replaced by pheophytin (Pheo) *a* ($\Phi_{A,B}$-exchanged RCs), shows a positive temperature dependence: it is 38 ± 5% between 10 and 60 K, increases with temperature to 72 ± 5% at 200 K and shows a minor additional increase above this temperature. The temperature dependence of the quantum yield of $P^+Q_A^-$ formation in $\Phi_{A,B}$-exchanged RCs is modelled in the framework of a reaction scheme with the energy level of $P^+Pheo_A^-$ placed above $P^+B_A^-$ (Shkuropatov, A.Ya. and Shuvalov, V.A. (1993) FEBS Lett. 322, 168–172), by the introduction of direct electron transfer from $B_A^-$ to $Q_A$, assisted by a superexchange-mechanism via $P^+Pheo_A^-$. The observed triplet formation of $\Phi_{A,B}$-exchanged RCs with pre-reduced $Q_A$ at cryogenic temperatures (quantum yield ≤ 12%) is attributed to a residual fraction of RCs in which only $\Phi_B$ was exchanged for Pheo *a*. The lack of triplet formation in pre-reduced $\Phi_{A,B}$-exchanged RCs is consistent with our kinetic model, since this predicts that at low temperatures the state $P^+Pheo_A^-$ is not populated. © 1997 Elsevier Science B.V.

*Keywords:* Triplet state; Pheophytin *a*; Pigment modification; Quantum yield; (*Rhodobacter sphaeroides*)

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## 1. Introduction

In part I of this publication [1] a spectroscopic characterisation was presented of reaction centers (RCs) of *Rhodobacter* (*Rb.*) sphaeroides R-26, in which the bacteriopheophytins (BPheos) *a* in both the active ($\Phi_A$) and the inactive branch ($\Phi_B$) were replaced by pheophytin (Pheo) *a*. These reconstituted RCs, referred to as $\Phi_{A,B}$-exchanged RCs, were obtained by a modified version [1] of the pigment-ex-

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Abbreviations: B, accessory bacteriochlorophyll; BChl, bacteriochlorophyll; (B)Pheo, (bacterio-)pheophytin; FWHM, full width at half maximum; I, intermediate electron carrier; P, primary donor; $^3P$, primary donor triplet; Q, acceptor quinone; RC, reaction center; T − S, triplet-minus-singlet; $\Phi$, bacteriopheophytin *a* electron acceptor

[*] Corresponding author. Fax: +31 71 5275819; E-mail: aartsma@biophys.leidenuniv.nl

*PII* S0005-2728(97)00039-X

change procedure reported by Scheer et al. [2]. Using our modified method we succeeded also in obtaining $\Phi_B$-exchanged RCs, in which only the BPheo in the inactive chain was replaced by Pheo *a* [1].

Recently, kinetic measurements have shown [3–5] that $\Phi_{A,B}$-exchanged RCs are photochemically active at room temperature. Two kinetic models were proposed to describe room temperature electron transfer in $\Phi_{A,B}$-exchanged RCs in terms of stepwise electron transport [3,4]. In contrast to the situation in native RCs, where the energy level of $P^+\Phi_A^-$ is located well below $P^+B_A^-$, both models place the level of $P^+Pheo_A^-$ in close proximity to that of $P^+B_A^-$. Shkuropatov and Shuvalov [3] suggested the energy level of $P^+Pheo_A^-$ to be slightly above $P^+B_A^-$, whereas Schmidt et al. [4] place it just below this level. A kinetic model including direct electron transport from $B_A^-$ to $Q_A$ via a superexchange mechanism has recently been suggested by Kirmaier et al. [6] for RCs of the so-called $\beta$-mutant, in which $\Phi_A$ was replaced by a bacteriochlorophyll (BChl). These mutated RCs display some characteristics of electron transfer and overall photochemistry similar to those of $\Phi_{A,B}$-exchanged RCs.

The quantum yield of charge separation, defined as the probability that excitation leads to the formation of the charge-separated state $P^+Q_A^-$, is only 70–80% in $\Phi_{A,B}$-exchanged RCs at room temperature [3,4]. This quantum yield differs substantially from that of native RCs, which has been reported to be essentially 100% [7]. The models used to describe the electron transport in $\Phi_{A,B}$-exchanged RCs correctly reproduce this reduced quantum efficiency [3,4]. However, when these models are used to predict the quantum yield of charge separation at low temperatures, significant differences appear. The temperature dependence of the quantum yield of $P^+Q_A^-$ formation is, therefore, an important criterion to assess the validity of particular assumptions in the kinetic models for electron transport in $\Phi_{A,B}$-exchanged RCs. We report here the results of such measurements on $\Phi_{A,B}$-exchanged RCs, and compare these with the predictions of several models. A reasonable fit of the temperature dependence was obtained with the model of Shkuropatov and Shuvalov [3], after the introduction of direct electron transfer from $B_A^-$ to $Q_A$, assisted by $P^+Pheo_A^-$ via a superexchange mechanism.

The temperature dependence of the quantum yield of formation of the primary donor triplet state ($^3P$) in pre-reduced $\Phi_{A,B}$-exchanged RCs relative to that in native RCs was also determined. This data provides a further test for the models describing electron transfer in $\Phi_{A,B}$-exchanged RCs. Our kinetic model predicts that no triplet state is generated in pre-reduced $\Phi_{A,B}$-exchanged RCs and that the triplet state formation is due to a residual fraction of up to 10% of $\Phi_B$-exchanged RCs. The corresponding triplet-minus-singlet $(T-S)$ spectra and triplet halftimes have been characterised, from which it is concluded that in the $\Phi_B$-exchanged fraction neither the structure of P, nor the (dipolar) interactions of P with the accessory BChls are changed substantially by the pigment exchange procedure.

## 2. Materials and methods

RCs of *Rb. sphaeroides* R-26 were isolated as described in [8] and [9]. The $\Phi_{A,B}$ molecules of these RCs were replaced by Pheo *a*, using a slight modification of the procedure developed by Scheer and co-workers [2,10,11]; see Part I of this publication [1]. The pigment exchange was better than 95% [1]. In order to ascertain that all RCs have a functionally bound $Q_A$, the RCs were reconstituted with synthetic ubiquinone-10 (Sigma), added in a 10:1 quinone to RC ratio. To obtain a clear glass at low temperatures, glycerol was added to all samples to a final concentration of $65 \pm 5\%$ (v/v). Absorbance difference spectroscopy was performed in the presence of 5 mM ortho-phenanthroline to inhibit $P^+Q_B^-$ state formation. Triplet state measurements were carried out using samples with pre-reduced $Q_A$, obtained by freezing the sample under continuous illumination in the presence of 75 mM sodium ascorbate.

Flash-induced absorption-difference kinetics were measured using a single-beam spectrophotometer, with excitation flashes ($\sim$ 10 ns FWHM) provided by a Nd-YAG laser or by a tunable optical parametric oscillator (OPO) pumped with a Nd-YAG laser. The measurements were performed in a helium-flow cryostat, using cuvettes with an optical pathlength of 1 mm. The time resolution in the kinetic measurements was determined by the setting of a low-pass filter (1 kHz–1 MHz), of which the actual value depended on the experimental requirements. The absorption difference spectra (Fig. 2) were obtained by plotting the value of the absorbance change at time $t = 0$ at each wavelength, determined by extrapolating a mono-exponential fit of the kinetics.

The absolute quantum yield of $P^+Q_A^-$ formation in $\Phi_{A,B}$-exchanged RCs was determined at each temperature with respect to that in native RCs, the quantum yield of which is reported to be 100 ± 15% over the full temperature range of 10–300 K [12]. The quantum yield in $\Phi_{A,B}$-exchanged RCs was obtained by measuring the ratio of the amplitude of the absorbance decrease at the P-band caused by $P^+$ formation in $\Phi_{A,B}$-exchanged RCs, to that in native RCs, under identical experimental conditions. The absorbance changes were induced by non-saturating flash excitation ($< 3\%$ of the RCs are excited) and their amplitude was determined by extrapolating of a fit of the decay kinetics to $t = 0$. For all measurements the same excitation wavelength of 880 nm was used, which was selected taking into account the shift of the long-wavelength band of P from approximately 865 nm at room temperature to about 897 nm at 10 K [1]. At each temperature, the wavelength of the absorption maximum of the P-band was used for detection (taken from [13]). Measurements were performed first for a sample containing native RCs over the complete temperature range, after which a second sample containing $\Phi_{A,B}$-exchanged RCs was inserted, using a HeNe laser for alignment to ensure identical sample positions and orientations. The two samples were adjusted to an optical density of 0.120 ± 0.005 at 865 nm at room temperature. Since the shape and the maximum position of the long-wavelength band of P for $\Phi_{A,B}$-exchanged and native RCs are nearly identical at 10 and 300 K [1], a similar temperature dependence of the optical characteristics of the P-band (shape, maximum position and absorbance) is expected for both types of RCs. To test the reproducibility of these measurements, the above mentioned ratio of the bleachings due to $P^+$ was determined in the temperature region of 10 to 100 K for two samples both containing native RCs. This measurement gave a value of 95 ± 5% over the temperature range used.

The quantum yield of $^3P$ state formation in $\Phi_{A,B}$-exchanged RCs, relative to that in native RCs, was obtained in a similar manner by measuring a ratio of the bleachings of the P-band due to $^3P$, for samples with pre-reduced $Q_A$.

## 3. Results

### 3.1. Temperature dependence of the quantum yield of $P^+Q_A^-$ formation

Fig. 1 (circles) shows the temperature dependence of the quantum yield of $P^+Q_A^-$ formation in $\Phi_{A,B}$-exchanged RCs. The inset gives an example of the kinetics of the absorbance changes from which the quantum yield was calculated (see Section 2). Despite the presence of 5 mM ortho-phenantroline, some $P^+Q_B^-$ formation occurred at temperatures above 220 K [14]. Taking into account that the $P^+Q_B^-$ recombination time is in the order of seconds [15], the repetition rate in experiments above 220 K was reduced to 0.3 Hz to avoid photo-accumulation of $P^+Q_B^-$. Since the amplitude of the absorption decrease of the P-band due to $P^+$ is the same for $P^+Q_A^-$ and $P^+Q_B^-$, formation of $P^+Q_B^-$ does not influence the measured quantum yield. The data was corrected for the presence of a residual fraction of 10% of $\Phi_B$-exchanged RCs (see also Section 4.2), assuming a $P^+Q_A^-$ yield of 100% in these RCs.

The quantum yield of $P^+Q_A^-$ formation in $\Phi_{A,B}$-exchanged RCs was found to be 38 ± 5% at 10 K (Fig. 1). This yield remained constant up to 60 K within the experimental uncertainty, and then increased with

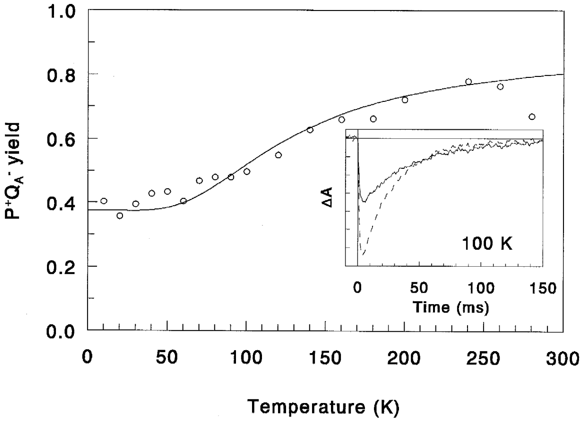

Fig. 1. Measurement (circles) and fit (line) of the temperature dependence of the quantum yield of $P^+Q_A^-$ state formation in $\Phi_{A,B}$-exchanged RCs, relative to that in native RCs. For the experimental conditions and fit-parameters (see Section 4). Inset: representative decay kinetics at 10 K for native (broken line) and $\Phi_{A,B}$-exchanged RCs (solid line), measured at 892 nm, upon non-saturating flash excitation at 880 nm.

temperature to 72 ± 5% at 200 K. Above this temperature only a minor increase was observed. The quantum yield for $P^+Q_A^-$ formation in $\Phi_{A,B}$-exchanged RCs at room temperature is in good agreement with earlier reports of 70–80% [3,4].

### 3.2. The triplet state of P

The temperature dependence of the quantum yield of $^3P$ formation of $\Phi_{A,B}$-exchanged RCs was determined in the temperature region of 10–100 K, relative to that of native RCs, in a similar manner as for $P^+Q_A^-$ state formation (see above). The relative quantum yield was determined as the ratio of the bleaching of the absorption band of P due to $^3P$ in $\Phi_{A,B}$-exchanged to those native RCs. The bleachings were induced by non-saturating excitation at 880 nm and detected at 890 nm. From the small residual bleaching due to $P^+$, observed in the decay kinetics after 1.5 ms, it was concluded that over 95% of the RCs contained pre-reduced $Q_A$ (data not shown), which is an important requirement for the correct determination of the $^3P$ yield.

In the temperature range of 10–100 K, the quantum yield of $^3P$ state formation for $\Phi_{A,B}$-exchanged RCs was found to be only 12 ± 3% of the yield for native RCs. This result is an upper limit of the actual $^3P$ yield for $\Phi_{A,B}$-exchanged RCs, in view of the presence of a small fraction of non-exchanged BPheo *a* ($\leq$ 5%, see [1]).

By comparing the bleachings for native RCs caused by $^3P$ formation (using pre-reduced RCs) with those due to $P^+Q_A^-$ formation (RCs in normal state), the absolute $^3P$ yield of native RCs was found to decrease with temperature from 70 ± 5% at 10 K to about 45 ± 5% at 120 K (data not shown), which is in good agreement with previous reports [16].

Fig. 2A,B show the flash-induced $T-S$ absorbance-difference spectra detected at 10 K for native (open circles and broken line) and $\Phi_{A,B}$-exchanged RCs (filled circles and solid line) with pre-reduced $Q_A$. In both cases, the spectra are characterised by a bleaching of the P absorption band at 895 nm, sharp oscillating features in the region 790–830 nm (Fig. 2B) and a broad absorbance increase in the 700–800 nm region. For native RCs some additional increase is observed around 770 nm, probably caused by minor changes in the BPheo absorption bands upon triplet state formation. The $T-S$ spectrum of native RCs agrees with the spectra previously obtained by absorbance-detected magnetic resonance [17,18]. In the $T-S$ spectrum of $\Phi_{A,B}$-exchanged

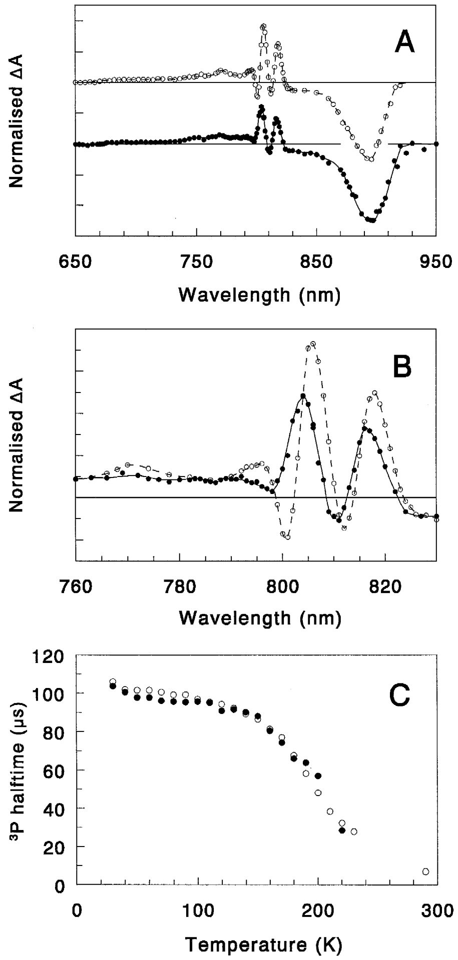

Fig. 2. A,B: triplet-minus-singlet absorbance difference spectra for photo-reduced native (open circles, broken line) and $\Phi_{A,B}$-exchanged RCs (filled circles, solid line) at 10 K upon non-saturating excitation at 890 and 760 nm (used for measurements in the region around 890 nm). The spectra were normalised at 890 nm. Conditions: spectral resolution $\leq 1$ nm for 650–850 nm and 2 nm for 850–950 nm. $\Delta A$ at 895 nm was $-0.03$ for native and $-0.005$ for $\Phi_{A,B}$-exchanged RCs. C: temperature dependence of $^3P$ decay halftime of native (open circles) and $\Phi_{A,B}$-exchanged RCs (filled circles) with pre-reduced $Q_A$, upon 532 nm excitation, measured in the presence of atmospheric oxygen. The detection wavelength for low-temperature measurements was 806 nm. The room-temperature measurement was performed using 865 nm as detection wavelength, in the presence of continuous illumination to keep $Q_A$ in its reduced state.

RCs the oscillations are blue-shifted by about 2 nm and the negative band at 802 nm is lacking. This shift corresponds to the blue-shift of the absorption band of B at this temperature [1]. The amplitude of the oscillations is diminished in the $T-S$ spectrum of $\Phi_{A,B}$-exchanged RCs, with respect to native RCs.

Fig. 2C shows that the $^3P$ state of native (open circles) and of $\Phi_{A,B}$-exchanged RCs (filled circles) have nearly identical decay halftimes at all temperatures in the range of 30–230 K. Previous reports showed a similar temperature dependence for native RCs [19,20]. Above about 200 K, measurements of the triplet state lifetimes are affected by quenching of the $^3P$ state by the oxygen present in the sample [20,21]. Moreover, above 220 K the pre-reduction of $Q_A$ by photo-accumulation was lost. For both native and $\Phi_{A,B}$-exchanged RCs, at temperatures below 30 K the triplet state decay kinetics could not be fitted using a single exponent, due to the thermal isolation of the $^3P$ spin-sublevels.

## 4. Discussion

### 4.1. Temperature dependence of the quantum yield of $P^+Q_A^-$ formation

The observation that at low temperatures the quantum yield of $P^+Q_A^-$ formation in $\Phi_{A,B}$-exchanged RCs is significantly lower than at higher temperatures (Fig. 1), immediately indicates that models in which activation-less stepwise electron transport is assumed and the free energy of the state $P^+Pheo_A^-$ is equal to, or below that of $P^+B_A^-$ [4], are not correct for $\Phi_{A,B}$-exchanged RCs at low temperatures. It could be argued that the introduction of a Gaussian distribution for the free energy of the state $P^+Pheo_A^-$ would improve the fit of the measured temperature dependence. In this way, in some fraction of RCs the $P^+Pheo_A^-$ state can be located above, and in another fraction below the $P^+B_A^-$ state. However, our calculations indicated that this adjustment to the model of Schmidt et al. [4] did not provide a satisfactory fit of the data (not shown). Several other adjustments to this model can be used to reproduce the temperature dependence of the $P^+Q_A^-$ yield, such as the introduction of a temperature dependence of the recombination rate from the state $P^+B_A^-$, the incorporation of a recombination channel via the state $P^+Pheo_A^-$ with a temperature-dependent rate, and a shift of the energy level of $P^+Pheo_A^-$ as a function of temperature. However, these ad-hoc assumptions cannot readily be justified by appropriate physical arguments.

A more realistic adjustment to the model of Schmidt et al. [4] is based on the assumption that the upward shift of the energy level of state $P^+Pheo_A^-$ in $\Phi_{A,B}$-exchanged RCs with respect to that of the $P^+\Phi_A^-$ state in native RCs [4], may lead to the formation of an energy barrier between the states $P^+B_A^-$ and $P^+Pheo_A^-$. In order to account for the non-zero quantum efficiency at low temperatures tunnelling through this barrier was assumed. This adjusted model indeed predicts the correct temperature dependence of $P^+Q_A^-$ formation, if a barrier of 450 $cm^{-1}$ and a tunnelling rate of 1.2 $ns^{-1}$ is used. However, at 10 K the quantum yield of $^3P$ formation (in pre-reduced RCs) predicted by this adjusted model is much higher than the observed yield of less than 12%. This can be seen qualitatively from the fact that in this model the yield of $P^+Pheo_A^-$ formation in pre-reduced RCs is similar to that of $P^+Q_A^-$ formation in open RCs. In turn, this implies that the predicted triplet yield for $\Phi_{A,B}$-exchanged RCs is about 40% of that of native RCs [16]. For this reason, also this adjustment of the model of [4] is not in agreement with the low-temperature measurements.

Another way to explain our results is to place the energy level of $P^+Pheo_A^-$ above that of $P^+B_A^-$, as has been suggested in [3]. Assuming stepwise electron transfer, this model will predict for low temperatures a quantum yield for charge separation of about zero. However, when direct electron transfer is introduced from $B_A^-$ to $Q_A$, assisted by $P^+Pheo_A^-$ via a superexchange mechanism, we arrive at a model that is in agreement with all experimental data. Fig. 3 shows the kinetic scheme of this model, which suggests that at low temperatures $P^+Pheo_A^-$ is not populated. The model is based on the idea that the quantum yield of $P^+B_A^-$ formation in $\Phi_{A,B}$-exchanged RCs is close to 100% ($k_{12} \gg k_{21}$, $k_{10}$), both at room [4] and at low temperatures, and that the differences in photochemistry of $\Phi_{A,B}$-exchanged RCs compared to native RCs are primarily determined by the changes in the kinetic properties of a '$P^+I^-$' state, to which both $P^+B_A^-$ and $P^+\Phi_A^-(P^+Pheo_A^-)$ contribute, caused by the replacement of BPheo by Pheo *a*.

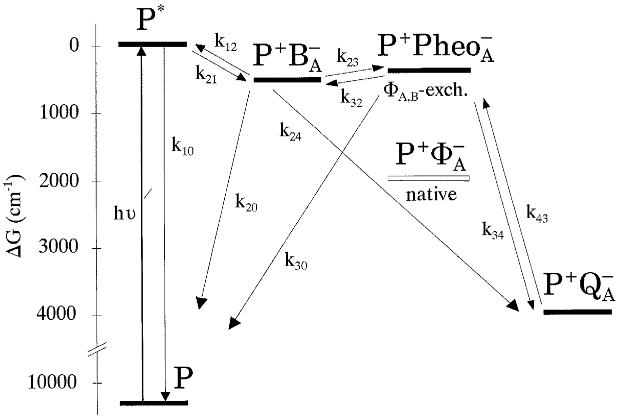

Fig. 3. Scheme of the kinetic model of electron transport in $\Phi_{A,B}$-exchanged RCs, used in this study. The details are given in the text.

The rate for the superexchange-assisted process $k_{24}$ can be determined at 10 K by taking into account the low-temperature $P^+Q_A^-$ quantum yield of 38% (Fig. 1). This quantum yield is given by $k_{24}/(k_{24}+k_{20})$, since at low temperatures the $P^+Pheo_A^-$ state is not populated. At room temperature, the rate of charge recombination of $P^+B_A^-$ to the ground state $(k_{20})$ has been determined to be 0.67 $ns^{-1}$ for $\Phi_{A,B}$-exchanged RCs with blocked electron transfer to $Q_A$ [3]. If this rate is assumed to be temperature-independent, the superexchange rate $k_{24}$ is about 0.41 $ns^{-1}$ at 10 K.

The energy difference between $P^+B_A^-$ and $P^+Pheo_A^-(\Delta E_{B\text{-}Pheo})$ in $\Phi_{A,B}$-exchanged RCs, can be derived from the value of the room-temperature time constant for electron transfer from $I^-$ to $Q_A$, by using the expression

$$k_{I-Q} = f_B k_{24} + f_{Pheo} k_{34} \tag{1}$$

valid for fast thermodynamic equilibration between the states $P^+B_A^-$ and $P^+Pheo_A^-$. Here $k_{I-Q}$ is the rate of electron transfer from $I^-$ to $Q_A$, $f_B$ and $f_{Pheo}$ are the populations of the states $P^+B_A^-$ and $P^+Pheo_A^-$, respectively, with $f_B + f_{Pheo} = 1$ and

$$f_{Pheo}/f_B = \exp(-\Delta E_{B-Pheo}/kT) \tag{2}$$

The rate constant $k_{I-Q}$ can be calculated from the observed $P^+I^-$ lifetime of 540 ps [3] (or 380 ps [4]). If one assumes that at room temperature $k_{34}$ is equal to the rate constant of 5 $ns^{-1}$ for electron transfer from $\Phi_A$ to $Q_A$ in native RCs [6], then one can find from Eqs. (1) and (2) that $f_{Pheo}/f_B = 0.43$ and $\Delta E_{B-Pheo} \approx 175$ $cm^{-1}$ at room temperature. From this energy barrier, it is possible to estimate the rate of direct electron from $B_A^-$ to $Q_A$ ($k_{24}$), by applying the standard expression for electron transport [22]:

$$k = \frac{2\pi}{\hbar} \cdot \frac{V^2}{\sqrt{4\pi\lambda kT}} \cdot e^{-(\lambda+\Delta G)^2/4\lambda kT} \tag{3}$$

First we use this expression to describe electron transfer from $B_A^-$ to $\Phi_A$ in native RCs. The energy gap $\Delta G_{B-\Phi}$ between these two states is obtained by subtracting the energy gap between $P^*$ and $P^+B_A^-(-450\ cm^{-1}$ [4,5]) from that between $P^*$ and $P^+\Phi_A^-(-2000\ cm^{-1}$ [23]). With $\Delta G_{B-\Phi} = 1550$ $cm^{-1}$ and $k_{B-\Phi} = 3$ $ps^{-1}$ obtained for native RCs at 10 K [24], and assuming the nuclear reorganization energy $\lambda$ to be equal to $\Delta G_{B-\Phi}$ (i.e. activationless electron transport), one finds for the electronic coupling term $V_{B-\Phi} = 32$ $cm^{-1}$. Assuming that this value also holds for $V_{B-Pheo}$ in $\Phi_{A,B}$-exchanged RCs, the approximation of Marcus [25] can be used:

$$V_{B-Q} = V_{B-Pheo} \cdot V_{Pheo-Q}/\Delta E_{B-Pheo} \tag{4}$$

where $V_{B-Q}$ is the electronic coupling term for the superexchange electron transfer from $B_A^-$ to $Q_A$. With $\Delta E_{B-Pheo} = 175$ $cm^{-1}$ (see above), we arrive at $V_{B-Q}/V_{Pheo-Q} = 0.18$. Furthermore, if we assume that the Franck–Condon factor for electron transport from $B_A^-$ to $Q_A$ is similar to that from $Pheo_A^-$ to $Q_A$, Eq. (3) gives $k_{B-Q}/k_{Pheo-Q} = (V_{B-Q}/V_{Pheo-Q})^2 = 0.033$. Taking $k_{Pheo-Q} = 10$ $ns^{-1}$ in $\Phi_{A,B}$-exchanged RCs at low temperatures, as for native RCs [6], we find that $k_{24} = k_{B-Q} = 0.33$ $ns^{-1}$ at 10 K. This value is in good agreement with that of 0.41 $ns^{-1}$, found from the quantum yield of $P^+Q_A^-$ formation at this temperature (see above).

Using the kinetic model of Fig. 3, and assuming fast thermal equilibration between $P^+B_A^-$ and $P^+Pheo_A^-$, the following expression for the quantum yield of $P^+Q_A^-$ formation $(\phi)$ is obtained:

$$\phi = \frac{f_B k_{24} + f_{Pheo} k_{34}}{f_B k_{24} + f_B k_{20} + f_{Pheo} k_{34} + f_{Pheo} k_{30}} \tag{5}$$

with $f_B$ and $f_{Pheo}$ defined as above. Fitting the measured temperature dependence of the quantum yield of $P^+Q_A^-$ formation with Eq. (5), we obtain optimal correspondence for $\Delta E_{B-Pheo} = 210$ $cm^{-1}$ and $k_{24} = 0.3$ $ns^{-1}$ (Fig. 1, solid line). The energy gap of 210 $cm^{-1}$ is slightly higher than the value of 175 $cm^{-1}$ obtained at room temperature. This difference may be a consequence of the temperature dependence of the parameters used, which is not incorporated in the calculations. Within this model we can also account for the observed yield of $^3P$ state formation at low temperatures (see Section 4.2).

The predominant characteristics of the model described above are similar to those of the model suggested by Kirmaier et al. [6] to describe electron transfer in $\beta$-mutant RCs of *Rb. sphaeroides*, in which $\Phi_A$ is replaced by BChl *a*. This is not surprising since these mutated RCs have several characteristics similar to those of $\Phi_{A,B}$-exchanged RCs, including an increased fluorescence intensity of $P^*$, an enhanced deactivation of $P^+I^-$ to the ground state, and a decrease of the quantum yield of $P^+Q_A^-$ formation upon cooling. However, Kirmaier et al. [6] suggested a varying rate of the superexchange-assisted process of electron transfer from $B_A^-$ to $Q_A$ to fit the temperature dependence of the quantum yield of $P^+Q_A^-$ formation in the $\beta$-mutant. Our experimental results for $\Phi_{A,B}$-exchanged RCs do not require such an assumption.

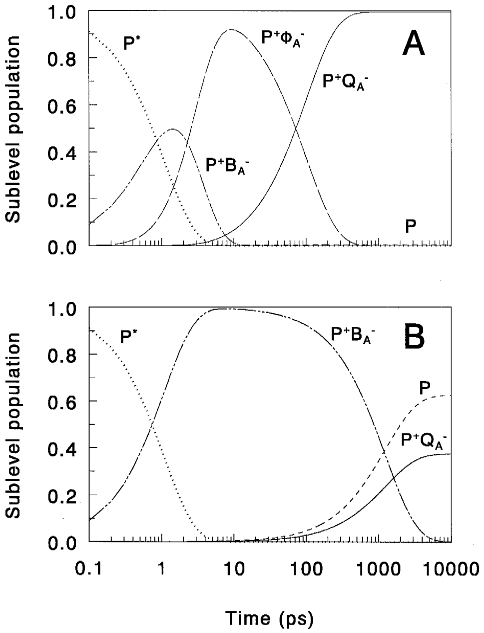

Fig. 4. Time-development of the sublevel populations at 10 K for native (A) and $\Phi_{A,B}$-exchanged RCs (B), calculated using the scheme presented in Fig. 3, with $k_{12} = 0.9$ $ps^{-1}$, $k_{20} = 0.5$ $ns^{-1}$ and $k_{24} = 0.3$ $ns^{-1}$ for native and $\Phi_{A,B}$-exchanged RCs. For native RCs $k_{23} = 0.5$ $ps^{-1}$, $k_{34} = 0.01$ $ps^{-1}$, and $k_{30} = 0.05$ $ns^{-1}$ was applied.

Using our model (Fig. 3), we can predict the time development of the sublevel populations at low temperatures for native and $\Phi_{A,B}$-exchanged RCs. The result of these calculations is presented in Fig. 4. Note that direct electron transfer from $B_A^-$ to $Q_A$ is also taken into account for native RCs. The predicted time developments for native RCs at 10 K (with $k_{34} = 10$ $ns^{-1}$ [6]) display a highly populated $P^+\Phi_A^-$ state and a much lower population of the $P^+B_A^-$ state. In contrast, the predicted time developments for $\Phi_{A,B}$-exchanged RCs show a long-lived, highly populated $P^+B_A^-$ state, without significant population of the $P^+Pheo_A^-$ state. Hence, according to our calculations, the low-temperature absorbance difference kinetics at 1020 nm due to absorption of the BChl anion [26,27] is expected to be quite different for $\Phi_{A,B}$-exchanged and native RCs, as was already observed at room temperature [4]. Obviously, low-temperature picosecond measurements (in progress) will provide a clear test for the validity of the assumed models.

## *4.2. The triplet state of P*

The quantum yield of $^3P$ formation in $\Phi_{A,B}$-exchanged RCs is only 12% of that in native RCs, at least at temperatures below 100 K, in agreement with earlier reports which also suggested a reduced triplet yield in $\Phi_{A,B}$-exchanged RCs [28,29]. This result may be compared (see above) with that of the $\beta$-mutant, which is reported to have quantum yields of triplet formation of 5 and 9% at 295 and 77 K, respectively [6].

We have noted, however, that up to 5% of the BPheos *a* in the $\Phi_{A,B}$-exchanged RCs is not exchanged [1]. Since these BPheos *a* are predominantly located in the active branch [1], the sample of $\Phi_{A,B}$-exchanged RCs contains up to 10% $\Phi_B$-exchanged RCs. The triplet state characteristics, i.e., lifetime, yield and T–S spectrum, of $\Phi_B$-exchanged and native RCs are probably similar, since it may be assumed that the active branch is hardly affected by reconstitution of $\Phi_B$. Thus, the fraction of $\Phi_B$-exchanged RCs by itself may account for the observed triplet yield in '$\Phi_{A,B}$-exchanged RCs' of 12% relative to that in native RCs. In turn, this implies that in

$\Phi_{A,B}$-exchanged RCs the triplet yield is significantly less than 12%, or even negligible, at low temperature.

Such a low triplet quantum yield is consistent with the relative populations of the intermediate states and their lifetimes as shown in Fig. 4. First of all, it has been established that in purple bacteria the $^3P$ state originates from charge recombination from the radical pair state $P^+\Phi_A^-$ under conditions when $Q_A$ is not functional (pre-reduced or removed; for a review, see [30]). In the proposed model (Fig. 3) for electron transfer in $\Phi_{A,B}$-exchanged RCs, the corresponding intermediate state $P^+Pheo_A^-$ is not populated at suffiently low temperatures (cf. Fig. 4B) and does not contribute to triplet state formation. The exchange interaction J between P and $B_A$ is bound to be several orders of magnitude larger that between P and $\Phi_A$ [31]. Moreover, the lifetime of the state $P^+B_A^-$ in $\Phi_{A,B}$-exchanged RCs with pre-reduced $Q_A$ ($\tau_{P^+B^-} = 1.5$ ns) [3] is much shorter than that of $P^+\Phi_A^-$ in native RCs ($\tau_{P^+\Phi^-} = 20$ ns). The yield of $^3P$ is approximately given by $2Q^2\tau^2/(1+4(J^2+Q^2)\tau^2)$, where Q represents the rate of singlet-triplet conversion in the radical pair [32]. For $\tau_{P^+B^-}/\tau_{P^+\Phi^-} = 0.08$, $Q_{P^+B^-} \sim Q_{P^+\Phi^-}$, $J_{P^+\Phi^-} \sim Q$ and $J_{P^+B^-}/J_{P^+\Phi^-} = 10^2$–$10^3$, the triplet yield from $P^+B_A^-$ recombination is negligible compared to that from $P^+\Phi_A^-$ recombination.

We assign the observed $T-S$ spectrum and the triplet state lifetimes in $\Phi_{A,B}$-exchanged samples to the small fraction of $\Phi_B$-exchanged RCs. The observed triplet state halftime of this fraction is similar to that of native RCs over the complete temperature range of 10–220 K, as shown in Fig. 2C. Moreover, the observed $T-S$ spectrum is nearly identical to that of native RCs [18]. The minor differences can be attributed to the exchange of the BPheo *a* in the inactive branch. A small blue-shift of the bands of the accessory BChls of native RCs, corresponding to related shifts in the low-temperature absorption spectra [1], is sufficient to reproduce the $T-S$ spectrum of the fraction of $\Phi_B$-exchanged RCs, following the procedure of Vrieze and Hoff [18]. The presence of oscillations in the $T-S$ spectrum at 10 K of the fraction of $\Phi_B$-exchanged RCs similar to those of native RCs confirms that neither the configuration of P, nor the interactions of P and the accessory BChls are altered substantially by the pigment exchange procedure [1].

The results of a recent $^3P$ study of $\Phi_{A,B}$-exchanged RCs at low temperatures by means of flash-EPR techniques, showing a similar electron spin polarization pattern and zero-field splitting parameters $|D|$ and $|E|$ as in native RCs [28], may also have to be re-evaluated in view of our new interpretation. Since the $\Phi_{A,B}$-exchanged RCs used in the study of [28] contained some residual BPheo, the observed triplet EPR signal from $\Phi_{A,B}$-exchanged samples likely also originated from the fraction of $\Phi_B$-exchanged RCs. Its similarity to the native $^3P$ EPR spectrum confirms that there is little difference in the $^3P$ states of native and $\Phi_B$-exchanged RCs.

## Acknowledgements

This work was supported by the Netherlands Foundation for Chemical Research (SON), subsidised by the Netherlands Organisation for Scientific Research (NWO), and by the Russian Fund of Fundamental Research. A.Y.S. and V.A.S. acknowledge travel grants of NWO and INTAS. The authors thank S.J. Jansen for skilfully preparing the RCs and are grateful to J. Amesz for carefully reading the manuscript.

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