The study of the state [P870$^+$B800$^-$] in bacterial reaction centers by selective picosecond and low-temperature spectroscopies

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The selective picosecond excitation of *Rhodopseudomonas sphaeroides* (R-26) reaction centers (RCs) at 870 nm induces the formation of the transient state within <1 ps followed by the conversion into the state P^F ($P^{\cdot+}Bph^{\cdot-}$) during 7 ± 2 ps at both 293 K and 110 K. The transient state including the intense bleaching at 800 nm has been shown not to be due: (a) to photon excitation at 870 nm; (b) the excitation of P^+; (c) photoselection effects. The transient state is interpreted as the state $^1[P^+B^-]$ in agreement with earlier works. The primary formation of the state $^1[P^+B^-]$ and the big effective singlet–triplet splitting in this state correspond to the spectral splitting of the P band at 900 nm in R-26 RCs and at 1000 nm in *Rhodopseudomonas viridis* RCs found at 4.2 K and attributed to the optical transition to both ^1P and $^1[P^+B^-]$ states.

Selective picosecond spectroscopy *Bacterial reaction center* *Electron transfer* *Bacteriochlorophyll* *Bacteriopheophytin*

1. INTRODUCTION

Selective excitation at 880 nm of *Rhodospirillum rubrum* reaction centers (RCs) [1] induces within the first few picoseconds of excitation the bleaching of the bacteriochlorophyll (BChl) bands at 800 and 870 nm (the state $^1[P^+B^-]$, where P is the primary electron donor absorbing at 870 nm and B is BChl absorbing at 800 nm) without the changes of absorption of the bacteriopheophytin (Bph) bands. The relaxation of the 800-nm band is observed with kinetics close to the correlation function of the exciting and measuring Gaussian pulses with a duration of ~30 ps. This relaxation is accompanied by the bleaching of the Bph bands with the formation of the state [$P^{\cdot+}Bph^{\cdot-}$]. Sub-ps, non-selective measurements [2–4] have shown that the formation of the state $^1[P^+B^-]$ occurs within <1–2 ps followed by the conversion into the state [$P^{\cdot+}Bph^{\cdot-}$] with $\tau_e = 7 \pm 2$ ps. However, some experiments have shown the possibility of the transient kinetics of ΔA at 800 nm (the band of B) due to two photon processes upon non-selective (at 530 nm) [2] and even selective (at 880 nm) [5] excitation of RCs.

This work shows that the transient kinetics at 800 nm are observed at low intensities of excitation at 870 nm. The formation of the state $^1[P^+B^-]$ occurs within <1 ps on each absorbed photon, and each state $^1[P^+B^-]$ is converted into [$P^{\cdot+}Bph^{\cdot-}$] with $\tau_e = 7 \pm 2$ ps. The electron transfer from $Bph^{\cdot-}$ to quinone (Q_1) occurs for about 150 ps in agreement with [6,7].

The study of the singlet and triplet states in *Rhodopseudomonas sphaeroides* RCs has suggested the mixing of the locally excited states of P and possibly B with the state $^{1,3}[P^+B^-]$ [8]. These data as well as ps studies seem to demonstrate the existence of the charge transfer complex [PB] in RCs. Therefore one can expect the presence of $2e^-$ transitions:

$^1[P^+B^-] \leftarrow [PB]$ and $[^1PB] \leftarrow [PB]$ at 900 nm

separated by ~0.02 eV accordingly to the energy separation of the states denoted as $[^1PB]$ and

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$^1[P^+B^-]$ [8]. This work shows the expected splitting of the band at 900 nm in *Rps.sphaeroides* RCs and at 1000 nm in *Rps.viridis* RCs at 4.2 K into two bands separated by ~0.02 eV.

2. MATERIALS AND METHODS

RCs from *Rps.sphaeroides* (the blue-green R-26 strain) and *Rps.viridis* were isolated using the treatment of chromatophores with lauryldimethylamine-*N*-oxide and DEAE-cellulose chromatography. The measurements were made at 293, 110 and 4.2 K in water–glycerol (1:1, v/v) solutions.

To measure the absorbance changes with the selective excitation at 870 nm, the ps apparatus has been bulit up in a way similar to that in [1]. To improve the beam structure two apodized/spatial filters [10] were used before each amplifier. A mini-computer was used for signal aquisition, signal averaging and the excitation control. The excitation pulse at 870 nm, obtained in a parametric light generator, was 28 ± 3 ps in duration and ~0.5 mJ in energy. Attenuation of the excitation was used to avoid two photon processes. After 40 signal-averaging the sensitivity was $\sim 3 \times 10^{-3}$ absorbance and the time resolution was ~3 ps. The absorbance changes (ΔA) were measured when the polarization of the measuring light was parallel ($\Delta A^{\parallel}$) or perpendicular ($\Delta A^{\perp}$) to the exciting light.

3. RESULTS AND DISCUSSION

Fig.1 shows the kinetics of absorbance changes ($\Delta A^{\parallel}$) at 890 and 790 nm at 293 K in *Rps.sphaeroides* RCs excited at 870 nm. One can see the delay in the development of absorption at 790 nm with respect to the kinetics of the bleaching at 890 nm. The calculated curve (solid lines) [11] shows that this delay is 7 ± 2 ps in agreement with sub-ps data [2–4]. The same delay is also observed for $\Delta A^{\perp}$ at 750 nm (Bph band) (fig.1B). The absorbance increase can be seen at the beginning of $\Delta A^{\parallel}$ at 750 nm which is followed by bleaching (fig.1B) in agreement with earlier data [1]. This shows that there are at least two components of ΔA at 750 nm: one of which is related to the Bph band bleaching with negative polarization [12,13], and the other to the formation of the earlier state with positive polarization of absorbance increase at 750 nm.

Under the same conditions the transient kinetics at 798 nm are observed (fig.1C). The deconvolution [11] of the kinetics using the profile of picosecond pulses, shows (solid lines) that the rise-time of the transient state is <1 ps and the life-time is 7 ± 2 ps in agreement with the delay described above. The transient bleaching at 798 nm is observed for $\Delta A^{\parallel}$ and $\Delta A^{\perp}$ (fig.1C). The dichroism value of +0.2 is close to the dichroism value of the band at 800 nm (+0.3) obtained from photoselection measurements with lower excitation intensities necessary for correct photodichroism measurements [12,13]. If RCs are photooxidized before measurement, no transient bleaching at 798 nm is observed.

The transient kinetics at 798 nm is observed at low temperature (110 K), when it can be seen at low intensities of excitation with PRC equal 3 and 0.5 (PRC is number of photons/cross-section of RCs absorption at 870 nm) (fig.1D) and with the same rise-time ($\leqslant$1 ps) and the same lifetime (~7 ps). The light saturation curves for ΔA_{798} and ΔA_{870} are close to each other at 293 K and 110 K (not shown). It means that the transient kinetics at 798 nm is not due to the non-linear processes and each absorbed photon induces the formation of the transient state. Thus, the transient kinetics at 798 nm is not due to two photon processes, to the excitation of P^+, to the photoselection effects, and reflects the important step of the electron transfer in RCs.

Fig.2 (dashed line) shows the spectrum of ΔA measured at 110 K and at 85 ps after the center of the excitation pulses at 870 nm. This spectrum mainly corresponds to the spectrum of the state P^F ($[P^{\cdot+}Bph^{\cdot-}]$) in [1,6,7] and includes the bleaching of the P band at 870 nm and the Bph band at 750 nm and the blue shift of the band at 800 nm with the absorption increase at 790 nm. This spectrum includes some contribution (~30%) of the $[P^{\cdot+}Q_i^-]$ spectrum which only decreases the bleaching at 750 nm and gives an additional shoulder at 770 nm. The blue shift of the 800-nm band at low temperature is the same in P^F and $[P^{\cdot+}Q_i^-]$ spectra [8]. Fig.2 (solid line) shows the spectrum of ΔA measured at 110 K and at the delay corresponding to the maximal transient bleaching at 798 nm (ΔA°_{798}) after the subtraction of the mixing of the state P^F at this delay according to the following expression: $\Delta A_{\lambda} = \Delta A^{\circ}_{\lambda} - A^{85}_{\lambda}$.

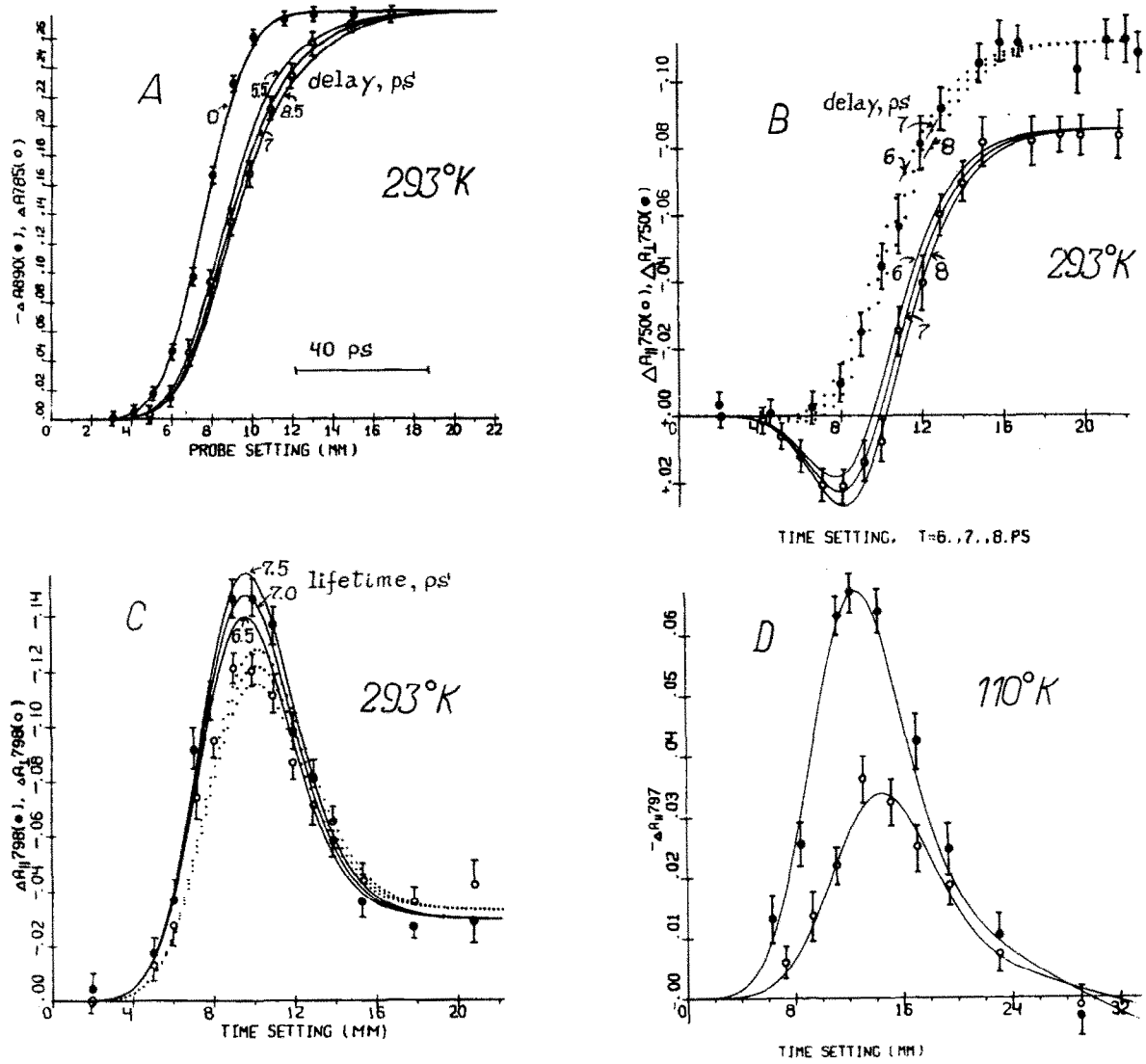

Fig.1. Picosecond kinetics of ΔA in *R.sphaeroides* RCs at 890 and 785 nm (A), 750 nm (B), 798 nm (C) and 797 nm (D), measured with parallel ($\Delta A^{\parallel}$) and perpendicular ($\Delta A^{\perp}$) orientation of *E*-vectors of the measuring and excitation at 870 nm beams. The number of photons/cross-section of RCs at 870 nm was 0.5 (○) and 3.0 (●) in fig.1D. Numbers show the delay and lifetime for calculated curves.

($\Delta A^{\circ}_{785}/\Delta A^{85}_{785}$), where ΔA° and ΔA^{85} are the absorbance changes measured at the center of the excitation and 85 ps after that, respectively. We suggest here that the $\Delta A^{\circ}_{785}/\Delta A^{85}_{785}$ ratio reflects the contribution coefficient of the state P^F at '0' delay according to the sub-ps measurements showing no absorbance changes at 785 nm at '0' delay [2–4]. Some contribution of the state $[P^{\cdot+}Q_i^{-}]$ at 85-ps delay only decreases the absorbance increase at 750 nm and 770 nm in the calculated spectrum of the transient state.

The transient state spectrum involves the bleaching at 798 nm and 880 nm and the development of the broad band with $\lambda < 780$ nm, the transient kinetics of which can be seen at 680 nm and 110 K (fig.2). The last ΔA shows also the component with $\tau_e = 150 \pm 20$ ps reflecting the electron transfer from $Bph^{\cdot-}$ to quinone in agreement with

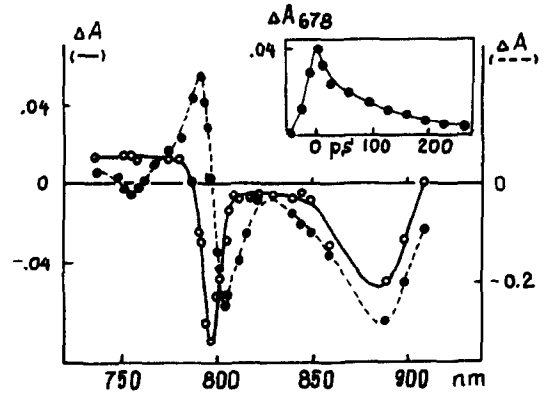

Fig.2. The spectrum of the state P^F in *R.sphaeroides* RCs measured at 110 K and at 85 ps after the center of the excitation pulse at 870 nm (– – –) and the spectrum of the transient state (——) measured at 110 K and at the delay of 8.5 mm (see fig.1D) after the correction and subtraction of the mixing of the state P^F at this delay. Insert shows the kinetics of ΔA at 568 nm (radical anion band) at 110 K.

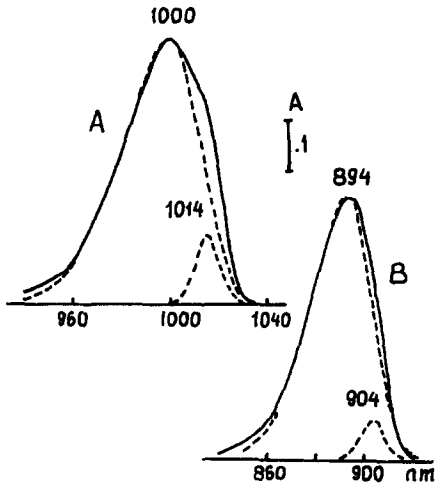

Fig.3. The absorption spectra of *Rps.viridis* (A) and *Rps.sphaeroides* (B) RCs at 4.2 K in the region of P band: (– – –) deconvolution of the spectra into the Gaussian bands.

[1,6,7]. The spectrum of the transient state is similar to that measured at ~ −15 ps before the excitation pulse center for *Rsp.rubrum* RCs [1]. The spectrum of the transient state can be interpreted in terms of the formation of the state $^1[P^+B^-]$, because the strong bleaching at 798 nm is obviously not due to either the formation of the singlet state of B800 upon one photon excitation at 870 nm or the formation of the triplet state of B within less than 1 ps.

Thus the formation of the state $^1[P^+B^-]$ occurs within less than 1 ps and is converted into the state $[P^{\cdot+}Bph^{\cdot-}]$ in 7 ± 2 ps at both 293 K and 110 K. The formation of the last state is accompanied by the bleaching of the Bph bands and blue shift of the B band with absorbance increase at 790 nm, the kinetics of which is delayed by ~7 ps with respect to the bleaching at 890 nm (fig.1).

The fast and primary formation of the state $^1[P^+B^-]$ as well as the large (~0.35 eV) effective singlet–triplet splitting in this state [8] suggest the existence of the electron-donor–acceptor complex [PB] with two transitions:

$$[^1PB] \leftarrow [PB] \text{ and } ^1[P^+B^-] \leftarrow [PB]$$

separated by ~0.02 eV in agreement with the energy separation between two levels including the mixing of the states $[^1PB]$ and $^1[P^+B^-]$ [8]. Fig.3 shows the expected splitting of Q_y band of P at 4.2 K into two bands separated by 0.02 eV in *Rps.sphaeroides* and *Rps.viridis* RCs. The similar splitting for *Rps.viridis* RCs has been described [16]. The fluorescence measurements [9] have shown that these bands reflect two electron transitions, the longest of which is the transition to the state $^1[P^+B^-]$ since it is very sensitive to the charge on the Bph molecule.

The close energy position of the transition $[^1PB] \leftarrow [PB]$ (1.36 eV in *Rps.sphaeroides* RCs) to the transition $^1[P^+B^-] \leftarrow [PB]$ is provided by the redox potentials of P^+/P (0.45 V [17]) and of BChl *a*/$BChl^-$ *a* (−0.86 V [18]) which give the energy of 1.31 eV for the transition $^1[P^+B^-] \leftarrow [PB]$, if $E_{1/2}$ for B/B^- and $BChl/BChl^-$ are about equal [19].

The existence of the electron-donor–acceptor complex [PB] promoted by the RC structure, and the mixing of the states $[^{1,3}PB]$ and $^{1,3}[P^+B^-]$, provide the following points which are important for the effective light energy conversion in the photosynthetic RC:

(i) The state $^1[P^+B^-]$ is obviously formed on each absorbed photon;

(ii) The state $^1[P^+B^-]$, storing ~98% of the energy of absorbed photon at 870–900 nm, is stabilized ($\tau_e \simeq 7$ ns in the absence of the electron transfer to Bph [8]) up to the lifetime of the excited state of BChl;

(iii) The state $^1[P^+B^-]$ has a constant dipole moment which oriented to the next electron carrier (Bph) by its negative wedge [20].

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