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Femtosecond spectroscopy of primary charge separation in modified reaction centers of *Rhodobacter sphaeroides* (R-26)

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Femtosecond measurements of kinetics and spectra of absorbance changes (ΔA) were carried out with modified reaction centers (RCs) from *Rhodobacter sphaeroides* (R-26) from which nonactive bacteriochlorophyll BM (located in the M protein subunit) was removed. The band of BM at 800 nm in native RCs is shifted in femtosecond measurements and obscures the ΔA of active bacteriochlorophyll BL (L subunit). The spectrum of ΔA in modified RCs at 6 ps delay includes the bleachings of the bands of P (primary electron donor) at 870 nm, of BL at 805 nm and of HL (bacteriopheophytin located in the L subunit) at 755 nm showing the reduction of ~0.5 mol BL and ~0.5 mol HL per mol P^+. These data confirm an earlier suggestion that BL participates as an electron acceptor in the light-induced primary charge separation and agree with recent X-ray analysis of *Rhodopseudomonas viridis* and *R. sphaeroides* RCs which shows a location of BL between P and HL.

Reaction center; Bacteriochlorophyll; Bacteriopheophytin; Charge separation

1. INTRODUCTION

X-ray analysis of reaction centers (RCs) and data on the amino acid sequence of protein subunits of RCs of *Rhodopseudomonas viridis* [1] have provided information about the pigment arrangement in RCs (see also [2,3]) and its connection with three-dimensional structure of protein subunits. Six molecules of pigments (four BChl and two Bph) and two quinones (Q) form two prosthetic chains: P-BL-HL-QL and P-BM-HM-QM, where P is the primary electron donor, BL and BM are BChl 'monomers', and HL and HM are Bph molecules located in the L and M protein subunits of RCs, respectively. Only the first chain, P-BL-HL-QL, is photochemically active [4].

To study the primary reactions in RCs occurring in the time domain of 100 fs to 1 ns, laser picosecond and femtosecond spectroscopy is used [5-15]. The latter renders correct data on the primary reactions in RCs since the pulse duration is less than the time constants of the reactions.

The interpretation of picosecond and femtosecond measurements [5-17] of the kinetics and spectra of absorbance changes (ΔA) includes at least three different suggestions:

(i) The primary state with separated charges is a state P^+BL^- which transfers an electron to HL [6,7,15];

(ii) The primary ionic state is P^+HL^-. The BL molecule does not participate in the charge separation but ΔA in its absorption band is influenced by the formation of P^+ [9,12];

(iii) The BL molecule participates in the electron transfer (at least at room temperature) but the

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Abbreviations: BChl, bacteriochlorophyll; Bph or H, bacteriopheophytin; BL and BM, BChl molecules located in L and M protein subunits of reaction centers, respectively; HL and HM, Bph located in L and M subunits, respectively

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primary ionic state includes the mixing of P^+BL^- and P^+HL^- [8,14,16].

The latter assumption is based on the simultaneous bleachings of BL and HL bands in femtosecond and nanosecond measurements [8,16]. The relative amplitudes of these bleachings are changed and transient kinetics of the BL band occur when RCs absorb a second photon at 880 nm [6,17].

Interpretation of the results obtained with intact RCs is complicated by the presence in these RCs of BM which does not participate in the charge separation, although its absorbance band is shifted by the formation of P^+ [4].

This paper presents the results of femtosecond measurements of kinetics and spectra of ΔA in *Rhodobacter sphaeroides* RCs which have the same arrangement of prosthetic groups as in *R. viridis* RCs [18]. To avoid the spectral features of BM induced by P^+, the BM molecule was removed by borohydride treatment [4].

2. MATERIALS AND METHODS

RCs from *R. sphaeroides* (R-26) were isolated and modified with borohydride as in [4]. Before measurements RCs were purified using DEAE-cellulose chromotography.

Measurements of the kinetics and spectra of ΔA were performed by means of the femtosecond laser double-beam spectrometer described in [19]. RCs with an absorbance of 1.2 at 870 nm were excited by ~300 fs pulses at 620 nm. Measuring pulses of the same duration in the 700–900 nm region were obtained by focusing of 620-nm pulses in a water cell. Each point of ΔA was obtained by averaging more than 50 measurements. The light curve for ΔA at 870 nm was measured at 0.6 ps delay of the measuring pulses with respect to the exciting pulses when the excitation of RCs was almost complete (fig.1). The intensity of exciting pulses was used in further experiments to excite less than 10% of the RCs (the only exception was for kinetic measurements at 755 nm when ~30% of RCs were excited to obtain a satisfactory signal/noise ratio).

3. RESULTS

The difference absorption spectra of modified RCs for various delays (fig.2) are somewhat

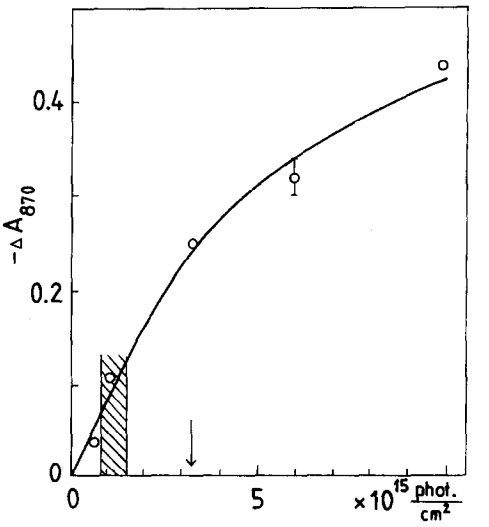

Fig.1. Light curve for ΔA at 870 nm measured in modified *R. sphaeroides* RCs excited by 300-fs pulses at 620 nm. Light intensities in the region of $0.8\text{–}1.5\times10^{15}$ photons/cm² were used for measurements presented in figs. 2, 3 except the kinetics at 755 nm which were measured using about 3.2×10^{15} photons/cm². Room temperature. Quinone acceptors were kept in the oxidized state. Absorbance at 870 nm was 1.2.

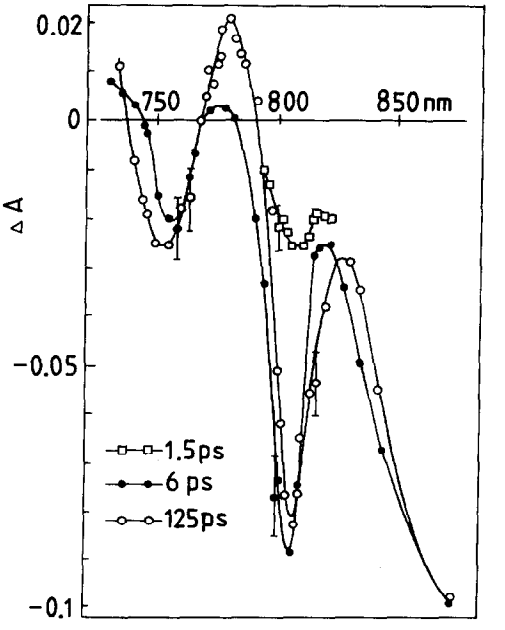

Fig.2. Difference absorption spectra of RCs measured at various delays. Conditions as for fig.1.

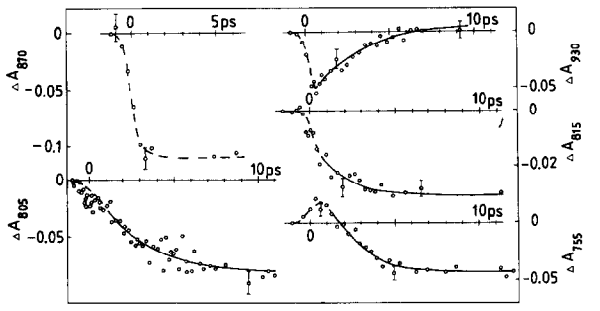

Fig.3. Kinetics of absorbance changes at 870 nm (P band), 805 and 815 nm (BL bands), 755 nm (HL band) and 930 nm (stimulated emission from P*). Conditions as for fig.1.

similar to those obtained for intact RCs under the same conditions [8]. In both cases, the bleaching of the P band at 870 nm, H band at 760 nm and B band at 805 nm is observed. However, at 6 ps delay (charge separation almost complete in the pigment part of RCs) only bleaching at 805 nm is observed in modified RCs in contrast to native RCs where an additional absorbance increase is observed at 780 nm. The latter is characteristic of the shift of the BM band induced by the formation of P^+ in intact RCs [4,8]. The shift of the BL band at 805 nm with an absorbance increase at 780 nm is observed at 125 ps delay when an electron is partially transferred to QL. Similar absorbance changes were observed at 805 and 780 nm in picosecond measurements for modified RCs [15].

Fig.3 demonstrates the kinetics of ΔA at 870, 755, 805, 930 and 815 nm. The excitation of P occurs from -1 to 0.5 ps delay as judged from the kinetics at 870 nm (P band). The kinetics of the 870 nm bleaching corresponds to the autocorrelation function of the excitation pulse. At the same delay a relatively small excitation of B (ΔA at 805 nm) can be seen. The excited state of B has a lifetime close to the pulse duration [8,13,14] and is probably related to the time constant of the energy transfer from B* to P. At delays >0.5 ps no considerable change of ΔA at 870 nm is observed.

After 0.5 ps delay the kinetics measured at 755, 805, 815 and 930 nm reflect the charge separation between P and other pigments. This is accompanied by the decay of P* measured by stimulated emission at 930 nm [7–12] and by the bleachings of BL and HL bands at 805 and 755 nm, respectively. Experimental data for the delays >1 ps were fitted by exponential curves with the following lifetimes (τ_e): 2.3 ± 0.5 ps for ΔA at 755 nm, 3.1 ± 0.4 ps for ΔA at 805 nm, 2.8 ± 0.7 ps for ΔA at 930 nm and 1.8 ± 0.7 ps for ΔA at 815 nm.

4. DISCUSSION

It was shown that the modification of *R. sphaeroides* RCs by borohydride does not change the high efficiency of the electron transfer in RCs [4,15]. The time constant for the electron transfer in modified RCs measured in this work is close to that presented earlier [8,9,12] for intact RCs (see also [15]). It is reasonable to suggest that the modification does not change the mechanism of electron transfer. The absence of BM and of its bandshift allows one to observe that the bleaching of the BL band at 805 nm in the spectra of ΔA at 6 ps is not related mainly to the shift of the BL band. We assume that this bleaching is related to the formation of BL^- as suggested earlier [8,16]. This means that an electron is transferred from P* to HL as well as to BL. These data are not consistent with the statement that only the HL molecule is an electron acceptor from P* [9–12].

The time constant for the kinetics of deactivation of P* (ΔA at 930 nm) is close, within experimental error, to that for the electron transfer to HL (ΔA at 755 nm) and BL (ΔA at 805 nm). Some difference between the time constants for ΔA at 755 nm (2.3 ± 0.5 ps) and ΔA at 805 nm (3.1 ± 0.4 ps) can be due to the 3-fold increase in excitation intensity for the measurements at 755 nm (see section 2) or to a real situation when HL is reduced faster than BL. Then the kinetics of ΔA at 815 nm (1.8 ± 0.7 ps), which is faster than those at 805 nm, might reflect the band shift of BL when HL is reduced. Another explanation of the relatively fast kinetics of ΔA at 815 nm can be related to real fast kinetics of BL^- formation. This explanation would be consistent with previously published data for picosecond measurements [15] at earlier delay (-38 ps).

The atomic coordinates of the prosthetic groups for *R. viridis* RCs (kindly provided by Dr J. Deisenhofer) allow one to calculate the minimal distances between the macrocycles of P, BL and BH. These coordinates are close to those for *R. sphaeroides* RCs [18]. The minimal distances be-

tween P and BL (~5 Å), P and HL (~12 Å) and HL and BL (~4 Å) correspond to time constants for the maximal rate of an electron transfer of ~1 ps, ~10 μs and ~0.1 ps, respectively (see [20]). Therefore, the most simple consistency of structural and optical studies can be approached by the suggestion that an electron is transferred from P* to BL within 1-3 ps and then redistributed between BL and HL within several tenths of a picosecond. If one suggests that the phytol tail of P directed to HL (see [1]) serves as a mediator of the electron transfer [8,21], the direct reduction of HL from P* is not excluded. This possibility needs further studies.

Taking the above discussion into account one can consider three alternative models for the electron transfer in RCs:

$$P^* \xrightarrow{k_1} P^+BL^- \underset{k_3}{\overset{k_2}{\rightleftharpoons}} P^+HL^- \quad (1)$$

$$P^* \xrightarrow{k_1} P^+HL^- \underset{k_3}{\overset{k_2}{\rightleftharpoons}} P^+BL^- \quad (2)$$

$$P^+HL^- \xleftarrow{k_1} P^* \xrightarrow{k_2} P^+BL^- \quad (3)$$

In all three models the electron transfer leads to the electron distribution between HL and BL. Models 1 and 2 are consistent with experiments if $1/k_1 \simeq 3$ ps and $1/k_2 \simeq 1/k_3 \simeq 0.2$-$0.6$ ps. Model 3 suggests that $1/(k_1+k_2) \simeq 3$ ps. If one suggests that the extinction coefficient (ϵ) at 755 nm for HL is ~0.5 of that for P at 870 nm and ϵ at 805 nm for BL is ~1.4 of that for P at 870 nm [4], then the bleachings of HL and BL can correspond to reduction of ~0.5 mol HL and ~0.5 mol BL per mol P^+.

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