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Transient absorption studies of the reaction center of the photosynthetic bacterium *Rb. sphaeroides* R-26 in the blue spectral range

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Introduction

Photosynthetic reaction centers (RC) are mainly hydrophobic pigment-protein complexes in which transmembrane charge separation and stabilization processes occur driving all the subsequent chemistry of photosynthesis. After light excitation of the reaction center the reactions start from the singlet excited state $^1P^*$ of a primary electron donor P (a dimer of bacteriochlorophylls *a*) which decays in some picoseconds by ultrafast electron transfer to a primary electron acceptor $BPhe_L$ (bacteriopheophytin *a*), creating the primary radical pair $P^+BPhe_L^-$. After primary charge separation, the electron is transferred to a secondary electron acceptor, the ubiquinone Q_A, in about 200 ps. When Q_A is prereduced or removed, the forward electron transfer is blocked at the level of $BPhe_L^-$ and the primary radical pair decays by charge recombination. It has been calculated that the characteristic time of charge separation must be a few ps or less. Martin et al. (1986), for the first time demonstrated that in RC of *Rb. sphaeroides* R26 one-step charge separation takes place in 2.8±0.3 ps and that no evidence is found for a second, faster kinetics attributable to the reduction of $BChl_L$, the intermediate accessory molecule. The opposite conclusion was drawn from the experimental study by Holtzapfel et al. (1990). At selected wavelengths within the 800 nm absorption band the biphasic kinetics was found, with time constants of 3.5±0.4 ps attributed to creation of $BChl_L^-$ and 0.9±0.3 ps connected with reduction of $BPhe_L$. From different type measurements it was estimated that the forward energy transfer from the reduced $BPhe_L$ to Q_A occurs in 200±50 ps.

In this paper we report a study of the absorbance changes in the blue range of spectrum associated with the electron transfer in reaction center from *Rb. sphaeroides* R-26 with 120 fs time resolution. Due to direct excitation of Q_y absorption band of $BChl_L$ and $BChl_M$ at 800 nm and its instantaneous transfer to P, we could study absorption bands far from this wavelength.

Materials and methods

Isolated reaction centers were resuspended at 10 μM RCs in 10 ml of 10 mM Tris, pH 8.0, 0.1% LDAO, 0.1 mM Na-ascorbate. RCs suspension was continuously flowing through the excited volume of the sample cell at a rate up to 10 ml/min, which enabled the total exchange

of the excited volume within 50 ms. The sample cell (laboratory made) with the windows made of fused silica plates, has the absorption layer of 1 mm in thickness.

The method of transient absorption measurements with a time resolution of 120 fs has been described in detail by Naskręcki et al. (1999). Excitation flashes of 80 fs duration at 800 nm were provided by a laser (Spectra Physics) at a repetition rate of 100 Hz. CCD camera was used for absorption measurements in range from 400 – 680 nm. Analysis of the traces was based on the three exponential fit.

Results

Fig.1 shows a ground state absorption spectrum of photosynthetic purple bacteria *Rb. sphaeroides* reaction centers. The excitation and tested by us transient absorption wavelengths are indicated. The figure shows the main absorption bands and their attribution to the components of the photosynthetic membrane.

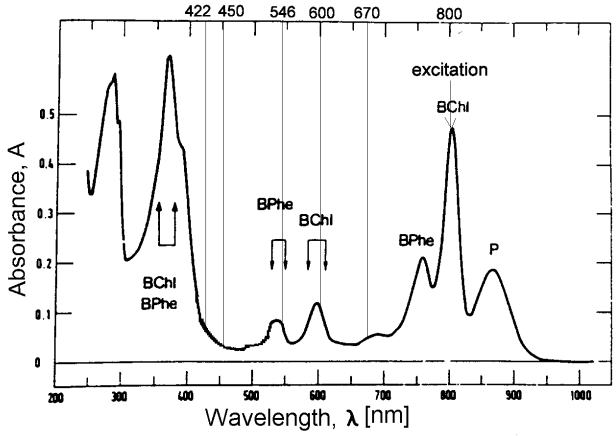

Fig.1. Absorption spectrum of photosynthetic purple bacteria *Rhodobacter sphaeroides* reaction centers. The excitation and transient absorption wavelengths are indicated

Fig. 2 shows the absorbance changes measured in the spectral region studied from 400 nm to 680 nm. The exemplary short delay times between the probe and the excitation pulse at 800 nm have been chosen as a) 1 ps, 2 ps, 5 ps and 10 ps, while the exemplary long delay times as b) 20 ps, 100 ps, 200 ps, 600ps.

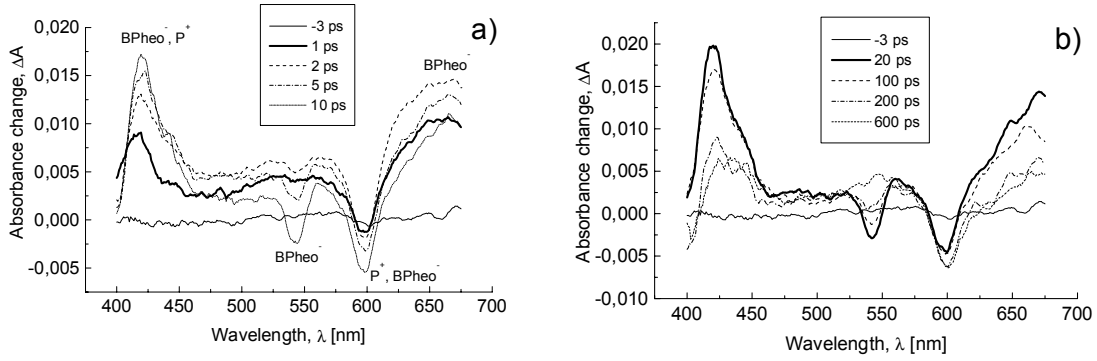

Fig.2. Absorbance changes observed in *Rb. sphaeroides* R-26 reaction centers at various delay times for: a) small range of delay time, b) large range of delay time

In both cases, the traces marked with –3 ps correspond to the signal obtained at 0 delay time. The difference spectra at these time intervals can be assigned to P^*, P^+, $BPhe_L^-$, and Q_A^-. The time evolution of the spectra reflects the excitation of the primary electron donor, $P \rightarrow P^*$, and the electron transfer reactions $P^* \rightarrow P^+BPhe_L^-$ and $P^+BPhe_L^- \rightarrow P^+Q_A^-$. To analyze the rates of the two latter reactions the absorption changes from 1 ps to 1 ns after excitation were averaged over 5 nm intervals and fitted with a constant plus three exponentials:

$$\Delta A_{fit} = A_0 + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3).$$

In this relation the time constant τ_1 reflecting the energy transfer reaction, $P \rightarrow P^*$ (exactly $PBChl \rightarrow PBChl^* \rightarrow P^*BChl$) was fixed to 120 fs due to the time resolution of our measuring system, τ_2 is the time constant for $P^* \rightarrow P^+BPhe_L^-$ electron transfer, τ_3 is the time constant of electron transfer from $BPhe_L^-$ to Q_A and t is the time after excitation. Using these time dependent absorbance changes, the kinetics discussed below are shown in Fig 3 and 4 for chosen wavelengths. They can be assigned to excited, oxidized and/or reduced cofactors participating in the electron transfer.

Fig. 3 shows the absorbance changes observed at 422 nm ascribed to four bacteriochlorophylls and to two bacteriopheophytins. After an initial instantaneous increase of absorption due to the creation of excited states of the bacteriochlorophylls in 120 fs, further absorbance changes are due to: a) creation of P^+ and $BPhe_L^-$ in $\tau_2 = 4.8 \pm 1.7$ ps and b) subsequent transfer of electron from bacteriopheophytin to quinone molecule in $\tau_3 = 250 \pm 90$ ps. The non-disappearing part of the signal is due to P^+.

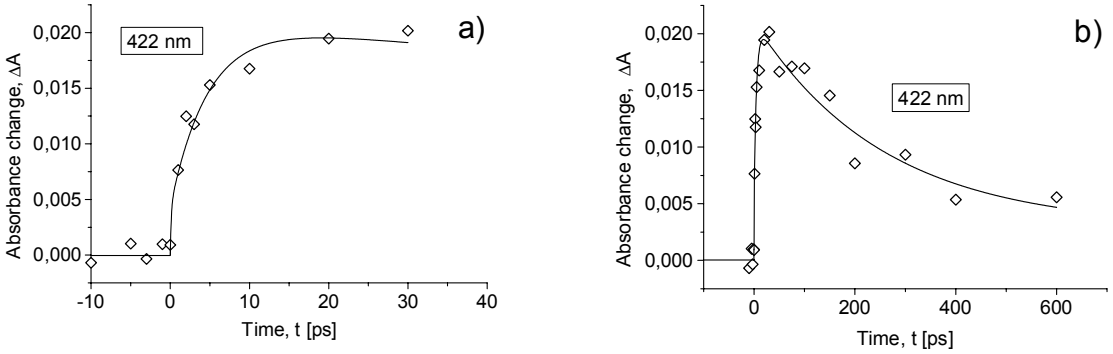

Fig.3. Kinetics of absorbance changes at 422 nm measured at room temperature for the reaction centers from *Rb. sphaeroides* R26 upon the excitation at 800 nm. a) in 40 ps time window, b) in 600 ps time window. The smooth curve represents the best fit to the observed kinetics. It includes the convolution of the pump and probe white pulse, with $\tau_1 = 120$ fs ($A_1 = -0.004 \pm 0.002$) response time of the apparatus, creation of P^+ and $BPhe_L^-$ in $\tau_2 = (4.8 \pm 1.7)$ ps ($A_2 = -0.016 \pm 0.002$) and subsequent transfer of electron from $BPhe_L^-$ to Q_A molecule in $\tau_3 = (250 \pm 90)$ ps ($A_3 = 0.018 \pm 0.003$); ($A_0 = 0.003 \pm 0.003$).

Discussion

In Fig. 4 the traces of the normalized absorbance changes measured in the blue range of the spectrum in 30 ps time window are presented for a few chosen wavelengths. The fits of all traces in this small time window can be obtained with two exponentials. The first exponential term of the fitting function corresponds to the instrumental rise time of τ_1=120 fs, whereas the second one depends on the wavelength.

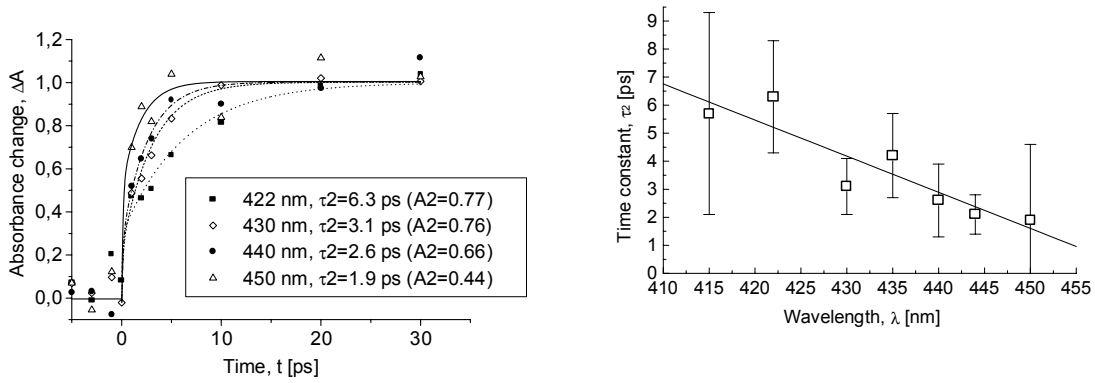

Fig. 4. (left) Kinetics of normalized absorbance changes in blue range of the spectrum measured at room temperature for reaction centers from *Rb. sphaeroides* R26 upon excitation at 800 nm in 30 ps time window. The traces were fitted with the two exponential function: $\Delta A_{fit} = 1 + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2)$ where τ_1 was fixed to 0.12 ps and $A_1 = 1 - A_2$.

Fig. 5. Wavelength dependence of τ_2 time constant in the blue range of the transient absorption spectrum.

The shorter the wavelength, the longer the τ_2 time constant, changing from 6.3 ps at 422 nm to 1.9 ps at 450 nm. The monotonic wavelength dependence of τ_2 time constant is shown in Fig. 5. A similar dependence of τ_2 was observed in the red region of the spectrum between 787 nm and 815 nm by Kirmaier and Holten (1990). The authors found that at room temperature the time constant for the initial charge separation and subsequent electron transfer to Q_A encompasses a range of values τ_2 = ~1.3-4 ps depending on the wavelength at which the kinetics is followed. It was suggested that this reflects a distribution of the RC (or a few conformers), differing in distances or orientations between the cofactors, hydrogen bonding, or other pigment-protein interactions. Consequently, different subpopulations of RCs are characterized both by slightly different spectral properties and primary charge separation kinetics. Our findings can be interpreted in the same way. The observed dispersion in τ_2 is also consistent with two theoretical models which take into account an intermediate state, P^+BChl^-: the "super-exchange" model and the three-step model. In the "super-exchange" model the electron immediately travels to the $BPhe_L$. $BChl_L$ functions as a virtual electron conductor. In the three-step model the electron is transferred in sequence from the excited special pair P^* to $BChl_L$, then to the $BPhe_L$, and further on to the quinon Q_A. Another model of primary charge separation proposes that the excited $BChl_L^*$ acts as the primary electron donor (Fischer and Scherer, 1987). An electron is transferred from $BChl_L^*$ to $BPhe_L$, giving $BChl_L^+BPhe_L^-$, and a subsequent charge transfer forms $P^+BChl_LBPhe_L^-Q_A$. Also light-induced rearrangements (Barzda et al. 1996) in RC driven by thermooptic effect can induce changes observed by us.

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