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PRIMARY CHARGE SEPARATION IN THE REACTION CENTERS OF RHODOBACTER SPHAEROIDES: EVIDENCE FOR A SEQUENTIAL ELECTRON TRANSFER VIA THE ACCESSORY BACTERIOCHLOROPHYLL

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The primary reaction of bacterial photosynthesis - an electron transfer via several prosthetic groups in the so-called reaction center - proceeds extremely rapid on the time-scale of picoseconds. A series of recent experiments gave the following picture of this charge transfer process (data taken for reaction centers from Rhodobacter (Rb.) sphaeroides /1-4/): After excitation of the lowest excited singlet state of the primary electron donor (a "special pair" of bacteriochlorophyll molecules) the excited electronic state P* lives for approximately 3.5ps. The decay of P* is related with the electron transfer away from P. From several time-resolved experiments it was concluded that this first charge transfer carries the electron directly to the bacteriopheophytin H /1,2/. Only very recently we could demonstrate the existence of an additional short-lived intermediate prior to the reduction of the bacteriopheophytin H /4/. We interpreted this intermediate as P^+B^-, i.e. the state where the electron from the special pair P has reduced the monomeric bacteriochlorophyll B to the anion radical B^-. In the final picosecond reaction the electron arrives (with a time constant of 200 ps) at the quinone Q_A. It is the purpose of this paper to present additional experimental data supporting a sequential electron transfer via the accessory bacteriochlorophyll.

Reaction centers from Rb. sphaeroides R26.1 and ATCC 17023 were prepared according to the procedure of Ref./4/. All experiments were performed at room temperature. In the time-resolved absorption measurements we used femtosecond exciting pulses ($t_p \approx 100$ fs, repetition rate 10 Hz) at 860 nm in the lowest energy absorption band of the special pair. Direct excitation of B or H via the exciting pulses could be ruled out. Probing of the light-induced absorption changes at various wavelengths, λ_{pr}, was performed with synchronized pulses (polarized parallel to the exciting pulses) as a function of the time delay, t_D. The excitation energy density was kept low. Less than 14% of the reaction centers were excited by each excitation pulse. Each of the experimental points presented here was averaged over more than one thousand single probing experiments.

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Absorption data measured as a function of delay time t_D between exciting and probing pulses are shown in Fig.1 for two wavelengths, λ_{pr} = 930 nm (Fig.1a) and λ_{pr} = 775 nm (Fig.1b). At 930 nm stimulated emission (gain) occurs. The signal is dominated by a strong absorption decrease decaying with a 3.5 ps time constant. The data do not exhibit any faster kinetic components. At the probing wavelength of 775 nm the transient absorption data contain additional information. At early delay times there is a pronounced absorption increase, which instantaneously follows the excitation process. Subsequently, a rapid relative absorption decrease is found until $t_D \approx 1$ ps. This transient has a time constant of 0.9 ps. Two additional time constants (3.5 ps and 200 ps) are required to fit the experimental points. These data are consistent with previous results /4/ suggesting that the reaction proceeds according to the following linear reaction scheme: Light absorp-

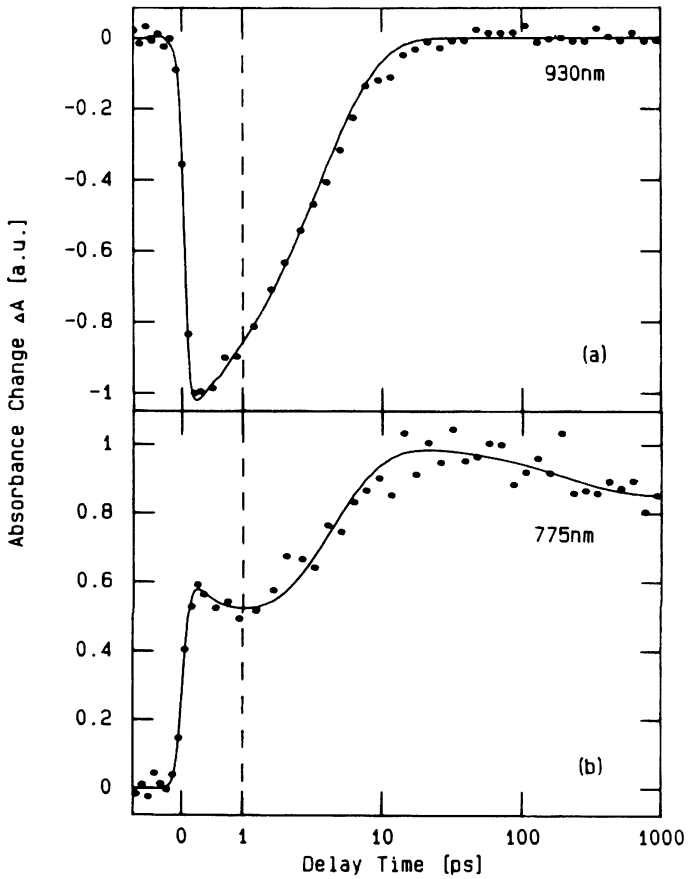

FIGURE 1. Time-resolved absorption data (points) for probing wavelength λ_{pr}=930 nm (Fig. 1.a, Rb. sphaeroides R26.1) and λ_{pr}=775 nm (Fig. 1.b, wild-type sphaeroides ATCC 17023). Excitation wavelength: 860 nm.

tion populates the excited electronic level P* (state I_1) which decays with a time constant τ_1 = 3.5 ps. It populates the subsequent state I_2 which has a shorter lifetime of 0.9 ps. The third intermediate I_3 contains a reduced bacteriopheophytin (state P^+H^-). It decays with the well-known time constant of 200 ps to intermediate I_4, where the electron has reached the quinone in state P^+Q^- /5/.

The solid curves of Fig.1 were calculated using the kinetic model given above. In Fig.1a no contribution by the 0.9 ps and the 200 ps kinetic is necessary to fit the experimental data. The decay of the gain is monoexponential with a 3.5 ps time constant. In Fig.1b a pronounced contribution of the 0.9 ps kinetic (in addition to the 3.5 ps and 200 ps decay) was necessary to obtain a satisfactory fit. The evaluation of the experimental data gives - besides the time constants - information on the amplitudes of the individual kinetic components. These amplitudes can be used to calculate the difference absorption cross-sections for the intermediates at the individual probing wavelengths /6/. This procedure, applied to a set of time-resolved data measured at various probing wavelengths between 720 nm and 970 nm, gave difference spectra for the intermediates. In Fig. 2 the difference

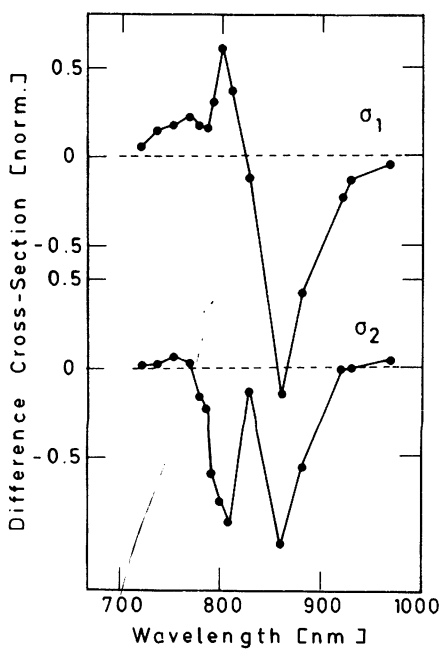

FIGURE 2. Difference spectra of the first two intermediates in the primary reaction of Rb. sphaeroides. The points are calculated from a series of transient absorption measurements. Upper spectrum: The 3.5 ps living excited electronic state P*. Lower spectrum: Second intermediate P^+B^-.

absorption cross-sections σ_1 and σ_2 of the intermediates I_1 and I_2 are shown as points (connected by the solid lines for better display). The excited electronic state P* has a difference spectrum (upper curve in Fig.2) with bleaching and/or stimulating emission at $\lambda > 820$ nm and excited-state absorption around 800 nm. The second intermediate I_2 shows a different spectrum. The absorption decrease around 860 nm is assigned to the disappearance of the special pair absorption in a state containing P^+. A second absorbance decrease occurs at 800 nm, where the accessory bacteriochlorophyll molecule initially absorbed. The amplitude of the cross-section decrease at 800 nm is consistent with the disappearance of the absorption of one monomeric bacteriochlorophyll due to the formation of state P^+B^-. This absorption decrease partially recovers with the formation of state I_3 (P^+H^-). Two additional experimental findings (not shown here) support the assignment of the configuration P^+B^- to state I_2: (i) Around 665 nm in the wavelength range where reduced bacteriochlorophyll molecules show a broad absorption increase, state I_2 also has strongly increased absorption. (ii) The angle between the transition moment of I_2 at 665 nm (determined in measurements using different polarisations of the probing pulses) and the Q_y transition of P agrees with estimations based on the pigment arrangement in the reaction centers /7,8/.

In conclusion: We have performed an improved experimental study of the primary charge transfer process in reaction centers of Rb. sphaeroides. The analysis of kinetic data and transient absorption spectra strongly suggest that the primary charge transfer from the special pair P to the bacteriopheophytin proceeds via the accessory bacteriochlorophyll as a true intermediate. The following stepwise reaction scheme results: After excitation of the special pair P an electron is transferred with a time constant of 3.5 ps to the accessory bacteriochlorophyll B. In the second step the electron proceeds with a time constant of 0.9 ps to the bacteriopheophytin H.

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