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GENERAL CONTENTS

CONTENTS TO VOLUME I

1. ANTENNA SYSTEMS IN PHOTOSYNTHETIC PROCARYOTES

4. PHOTOSYSTEM I

PRIMARY ELECTRON TRANSFER KINETICS IN BACTERIAL REACTION CENTERS WITH MODIFIED BACTERIOCHLOROPHYLLS AT THE MONOMERIC SITES $B_{A,B}$

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INTRODUCTION

In the primary charge separation of bacterial reaction centers (RC) an electron is transferred from the primary donor P to the acceptor, a quinone Q_A. The crystal structure [1-3] shows two intervening pigments i.e. a monomeric bacteriochlorophyll (BChl) B_A and a bacteriopheophytin (BPhe) H_A. There is general agreement that the electron reaches the BPhe H_A within 3-4 ps after excitation, generating the radical pair state $P^+H_A^-$. Two models are discussed for the transfer over the 10 Å edge to edge distance between P* and H_A. In the stepwise electron transfer (ET) model the BChl B_A is a real intermediate electron carrier, while in the superexchange model the electronic coupling between P* and H_A is promoted by the virtually populated radical pair states $P^+B_A^-$ [4, 5]. In early time resolved studies with sufficient temporal resolution and an appropriate excitation wavelength only one kinetic component was found favouring direct transfer to the BPhe H_A [6, 7]. The observation of an additional time constant of 0.9 ps in Rb. sphaeroides again raised the possibility of a stepwise ET according to [8, 9]:

$$P^* \xrightarrow{3.5ps} P^+B_A^- \xrightarrow{0.9ps} P^+H_A^- .$$

In the meantime several groups have found transient absorbance changes on a time scale shorter than $P^+H_A^-$ formation [10-14], however, the role of the monomeric BChl B_A is still discussed controversially. In this paper we present kinetic absorption studies on RCs containing [3-vinyl]-13^2-hydroxy-BChl a at the sites $B_{A,B}$ giving additional information on the primary electron transfer in native RC.

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MATERIAL AND METHODS

RCs from *Rb. sphaeroides* R26.1 were isolated as described in Ref. 15. RCs containing [3-vinyl]-13^2-hydroxy-BChl a were prepared after Struck et al. [15, 16]. Quinones lost during purification were reconstituted. The BChl a exchange yielded values of 40 ± 5 % for [3-vinyl]-13^2-hydroxy-BChl a. Since the two BChl a - molecules of the primary donor P do not exchange, this corresponds to an average exchange of 80 % at sites B_A and B_B.

Kinetic measurements were performed at $T \approx 298$ K in cuvettes of 1 mm path length under stirring. The sample volume was approximately 0.2 - 0.3 ml. The transmission was between $T \approx 10$ % and 50 % at $\lambda = 860$ nm. The experimental system has been described in detail in Ref. 16. Characteristics of the excitation pulses: $\lambda = 860$ nm or 875 nm ($\Delta\lambda$ = 20 nm), energy 1 µJ, duration 200 fs, spot size 1 mm (chosen to excite not more than 15 % of the RCs in the irriadiated volume by each laser pulse). Probing pulses: 20 nm wide portion of a femtosecond white light continuum ($t_{probe} \approx 200$ fs), parallel polarizations of exciting and probing pulses. The signal points (full circles) were modelled (solid curves) by a sum of exponentials convoluted with the experimental response function (for details see 9).

RESULTS AND DISCUSSION

The preparation of RCs containing the modified [3-vinyl]-13^2-hydroxy-BChl a shows a substantially different absorption spectrum from native RC (see insert in Fig. 1b). The absorption bands of the modified (monomeric) BChl's $B_{A,B}$ are shifted from 802 nm to 772 nm (Q_y(B)-band) and from 600 nm to 573 nm (Q_x(B)-band). However, around 801 nm, there is a shoulder in the shifted Q_y(B)-band. This shoulder is predominantly due to an incomplete exchange (80 %) of the monomeric BChl a. Part of this shoulder may also be due to the upper excitonic component of the Q_y(P)-band [15, 18, 19].

The decay of the electronically excited state P* is monitored in the gain-region at λ_{pr} = 920 nm (Fig. 1a). The best monoexponential fit is found for $\tau_1 \approx 17$ ps (dashed line in Fig. 1a). However, only a biexponential model function is able to trace the data reasonably well. When we use $\tau_1 = 3.5$ ps taking into account a fraction of unaltered RCs, we obtain the best simulation for a second time constant of $\tau_1' \approx 32$ ps (solid line in Fig. 1a) with an amplitude ratio of a_1(3.5ps): a_1'(32 ps) of approximately 1 : 2. In order to ascertain that the 32 ps - kinetic component does not describe a simple relaxation of P* back to the ground state P, we measured the transient absorption change at $\lambda_{pr} = 850$ nm (Fig. 1b) where the light induced absorption decrease is mainly due to the bleaching of the Q_y(P)-absorption. The high degree of bleaching at late delay times (t_D = 1 ns) excludes, that the 32 ps component is related to internal conversion. We conclude therefore that it reflects a real electron transfer step. Further probing wavelengths were chosen in the Q_y-absorption (770 nm to 801 nm) and in the anion bands of the BChls (645 - 665 nm) [20]. The results were: (i) The 32 ps component also appears in these spectral regions. (ii) The transfer rate to Q_A of 200 ps is not affected by the modification. (iii) There is no distinct 0.9 ps kinetic component.

The data on the [3-vinyl]-13^2-hydroxy RC preparation give further informations on the applicability of the reaction schemes for wild-type RC. In the superexchange model the fast kinetic component would be related to an S_1-relaxation of the excited special pair P*. This process, however, should not be affected by the exchange of the monomeric BChls. If the 0.9 ps component preceeds the 32 component it should show up clearly in the transient

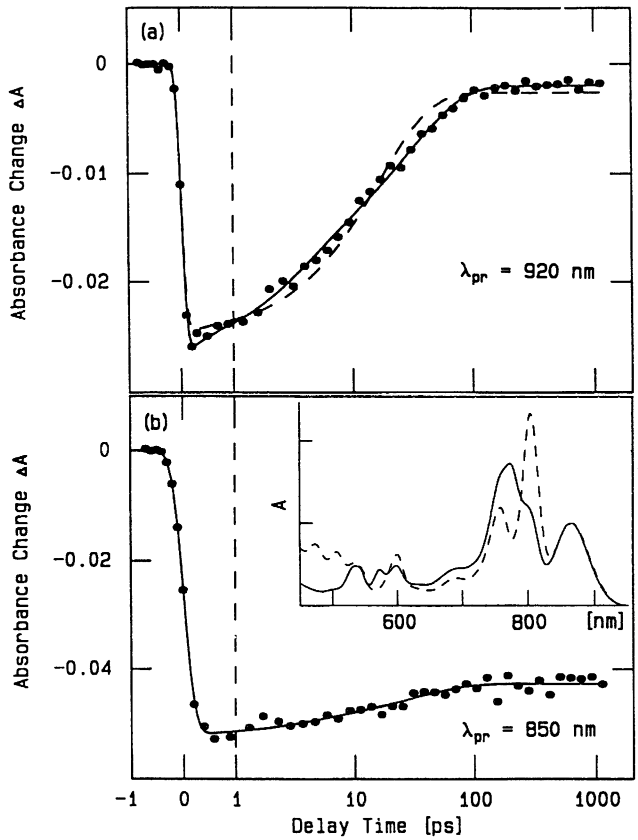

Fig. 1: Transient absorption data on the [3-vinyl]-13^2-OH-RC preparation (full circles): (a) probing wavelength, 920 nm, the dashed curve represents the best mono-exponential fit (time-constant) τ_1 = 17 ps); the solid line is a bi-exponential model function using time constants of τ_1 = 3.5 ps and τ'_1 = 32 ps (amplitude ratio a_1:a_1' = 1:2). (b) Probing wavelength of 850 nm. Insert: Absorption spectra of the [3-vinyl]-13^2-OH-RC preparation (solid line) and ATCC 17023 wild-type - RCs (broken line). The spectra are normalized to A(860 nm) = 1. Differences at $\lambda < 540$ nm are due to the absorption of a carotenoid present in the ATCC 17023 RCs.

absorption data. The apparent absence of a fast kinetic in the modified RCs supports the idea of a stepwise electron transfer in native RCs.

Assuming a life time of P* of 32 ps in the modified RCs a fast kinetic component is not detectable within our experimental accuracy if it is shorter than approximately 8 ps. There are some indications that there must be a related process in the 5 ps regime. At present we cannot decide whether a direct superexchange ET to H_A takes place in the [3-vinyl]-13^2-hydroxy RCs or if the reaction is still stepwise via $P^+B_A^-$. Most likely the reason for the change of the ET reaction is the rise in free energy of the radical pair state $P^+B_A^-$ due to the vinyl group replacing the acetyl group. This leads to a reduced speed of the primary reaction in both models.

In conclusion, in RCs with [3-vinyl]-13^2-hydroxy BChl a at sites B_A, B_B the primary charge separation is considerably decelerated. A detailed analysis of the data suggests a stepwise sequential electron transfer model for wild-type RCs.

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