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# Excitation relaxation in the chemically modified photosynthetic reaction center from *Rhodobacter sphaeroides* 601

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**Abstract** The ultrafast excitation relaxation in the sodium borohydride-treated reaction center of *Rhodobacter sphaeroides* 601 was investigated with selective excitation. From the femtosecond pump-probe measurement at 790 nm, the excitation relaxation demonstrates a biexponential decay with time constants of about 200 fs and 1.4 ps. By comparison with the result from sodium ascorbate-pretreated modified RS601, it could be concluded that the dynamical trace at 790 nm mainly originates from the contribution of accessory bacteriochlorophyll in the active side, and the electrochromic shift arising from the induced positive charge on the special pair primarily affects the absorption band in the red region of the accessory bacteriochlorophyll in RS601. With direct excitation of the special pair, the charge separation and subsequent electron transfer were observed in borohydride-modified RS601. The 2.8 ps component was ascribed to the charge separation and electron transfer from P* to $H_A$. From the dynamical traces at 790, 800 and 818 nm, the ultrafast energy relaxation from the excited accessory bacteriochlorophyll in the active side is consistent with a two-step energy transfer mechanism. This dynamical observation in modified RS601 is of significance in understanding the physical mechanism of excitation relaxation and energy transfer in the photosynthetic primary process.

**Keywords** Photosynthetic reaction center · Ultrafast dynamics · Energy transfer

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## Introduction

In the initial processes of photosynthesis, the excitation energy harvested by antenna complexes is trapped by a photosynthetic reaction center (RC), in which the conversion of excitation energy into electrochemical energy occurs and the electrochemical energy is ultimately used to drive chemical reactions. The RC from the purple photosynthetic bacteria *Rhodobacter sphaeroides* contains eight cofactors, two coupled bacteriochlorophylls known as the special pair (P), two accessory bacteriochlorophylls (B), two bacteriopheophytins (H), which are thought to be the primary electron acceptors, and two quinones (Q) (Michel and Diesenhofer 1989; Hu et al. 1998). These cofactors are arranged with approximate twofold symmetry around the axis into two branches (A-branch and B-branch), and each branch contains an accessory B ($B_A$ or $B_B$), a bacteriopheophytin H ($H_A$ or $H_B$) and a quinone Q ($Q_A$ or $Q_B$). The process and mechanism of energy transfer from the accessory bacteriochlorophyll to the special pair in isolated RCs of *Rb. sphaeroides*, upon excitation of the accessory bacteriochlorophylls, have been the subject of a number of investigations (Jia et al. 1995; Harn et al. 1996; Jonas et al. 1996; Stanley et al. 1996; Vulto et al. 1997). It was suggested that the energy transfer from B* to P occurs on a hundreds of femtoseconds or shorter time scale. Recently, Arnett et al. (1999) reported the wavelength-resolved pump-probe and anisotropy results of the *Rb. sphaeroides* R26 reaction center with the simultaneous excitation of accessory $B_A$ and $B_B$, and proposed a two-step mechanism of energy transfer within the RC, in which the energy transfer process is flowing sequentially from accessory B* to $P_{Y+}$ (upper excitonic state of P) to $P_{Y-}$ (lower excitonic state of P). It has been shown that the excitation of P, either directly or through excitation transfer from other pigments, can initiate the charge separation and a sequence of electron transfer reactions. However, it is generally accepted that only P and the cofactors most closely associated with the

A-branch (P, $B_A$, $H_A$, and $Q_A$) are involved in the transmembrane electron transfer process.

The RC modified by chemical treatment or pigment exchange is of considerable interest both for experimentalists and theoreticians (Vulto et al. 1997; King et al. 2000). It was previously noted that $B_A$ and $B_B$ do not absorb at exactly the same wavelength. $B_A$ has an absorption maximum at 802 nm and $B_B$ at 812 nm at low temperature. These bands are broadened at room temperature, but a difference in absorbance can still be found. The treatment of RC with borohydride provides a method to modify the spectral properties of RC, with which we can spectrally distinguish the $B_A$ and $B_B$ bands since the contribution of $B_B$ at 812 nm in borohydride-treated RCs can be fully removed (Ditson et al. 1984; Vulto et al. 1997).

In the present work, to obtain more information on the composition of the 800 nm band and the transient energy relaxation dynamics of the bacteriochlorophylls involved, we performed femtosecond pump-probe measurements on the modified RC RS601 isolated from *Rb. sphaeroides* 601 with selective excitation, especially investigating the relaxation process initiating from bacteriochlorophyll in the A-branch.

## Experimental

The growth of the bacterium *Rb. sphaeroides* 601 and the isolation of reaction center RS601 were reported previously (Zeng et al. 1997). The RS601 sample was dissolved in Tris-HCl buffer with 0.12 M NaCl, 10 mM Tris (pH 8.0) plus 0.025% *N*-lauryl-*N*,*N*-dimethylamine *N*-oxide (LDAO). Chemically modified RS601 was prepared by treatment of isolated RS601 with excess sodium borohydride as described (Shuvalov et al. 1986), and the pH of the modified RS601 was less than 10. To prohibit the accumulation of the special pair cation and keep the RS601 in a reduced state, sodium ascorbate was added to the sample in the pump-probe measurements at the excitation wavelength around 800 nm. The optical density of modified RS601 was set to 0.5 at 865 nm in a 1-mm cuvette. The one-color femtosecond pump-probe experiments were performed on a home-built system (Guo et al. 2001). A Ti:sapphire laser (Tsunami, Spectral Physics), pumped by a diode laser, served as the source of ultrashort pulses in our experiments. The output pulses were about 100 fs duration with a repetition rate of 82 MHz. The wavelength of the output pulses can be tuned from 750 to 870 nm with a 10 nm bandwidth (FWHM) by changing the position of the mode-locked slit across the beam profile. The pump pulse energy used in our measurements was less than 0.2 nJ with a beam diameter of about 50 μm in the beam overlapping region. This corresponded to a maximum photon flux of $5\times10^8$ photons per pulse. In the two-color pump-probe measurement, the output pulses from the Ti:sapphire regenerated amplifier laser (Spitfire, Spectral Physics), centered at 800 nm and with 130 fs time duration, were divided into two beams: one was used as the pump beam to excite the sample at 800 nm, and the other was used to generate white light from which a given wavelength was selected with an interference filter as the probe beam. All measurements in this work were carried out at room temperature.

## Results and discussion

It is known that the treatment of isolated RCs from *Rb. sphaeroides* with sodium borohydride leads to a partial loss of the 800 nm absorption band. Figure 1 shows the absorption spectra of chemically modified RS601 together with that of native RS601. The borohydride treatment still leaves the tetrapyrrole structures of the pigments intact and each RC contains two bacteriopheophytins and four bacteriochlorophylls (Struck et al. 1991). In comparison with Fig. 1a for native RS601, Fig. 1b for modified RS601 clearly shows that a reduction in absorbance for the 802 nm band, especially near 812 nm which mainly arises from $B_B$, is accompanied by an absorbance increase in the 720–780 nm region and a small decrease near 865 nm (Ditson et al. 1984; Vulto et al. 1997). If two bacteriochlorophylls ($B_A$ and $B_B$) contribute equally to the absorption at 800 nm, the measured absorption spectra indicate that $B_B$ has been removed to the most extent by the borohydride modification. The increase near 760 nm demonstrates the pheophytinization concomitantly. So it could be concluded that the $Q_Y$ absorption band of $B_A$ is shifted to the blue side of the 800 nm band in the modified RS601, and the effect of borohydride treatment is limited to the inactive branch. A small concomitant blue shift of the P absorption band mainly arises from environmental effects, indicating that there is specific reaction of borohydride with the protein. It has been confirmed that borohydride treatment is unlikely to significantly modify the protein structure and the primary electron transport is essentially unaffected in these chemically modified RCs (Ditson et al. 1984; Chekalln et al. 1987; Vulto et al. 1997).

In the femtosecond pump-probe measurements, we defined the pump-induced increase of transmission (bleaching/stimulated emission) as a positive pump-probe signal. The transient transmission change of the chemically modified RS601 and of the pretreated (with sodium ascorbate) modified RS601 upon 800 nm pulse excitation with a parallel polarization pump-probe configuration are shown in Fig. 2. In the first hundreds of femtoseconds after the zero time point, both the

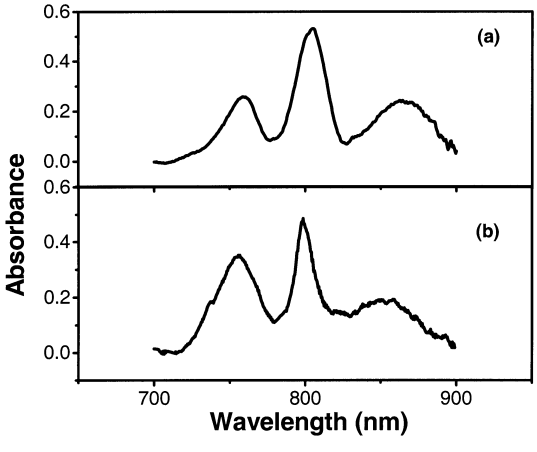


**Fig. 1** The absorption spectra of (**a**) native RS601 and (**b**) chemically modified RS601 reaction centers from *Rb. sphaeroides* 601

traces are characterized by an ultrafast decay of bleaching with a pulse-duration limited time scale. For borohydride-modified RS601, biexponential fitting of the trace leads to two components with about 240 fs and 1.6 ps time constants. For ascorbate-pretreated modified RS601, however, the initial rapid decay is followed by a partial bleaching recovery within several picoseconds. In previous reports the ultrafast energy transfer from B* to P has been confirmed many times in various experiments, which takes place in hundreds of femtoseconds or an even shorter time domain, depending on the bacterial species and techniques used. It is generally suggested that the excitation energy from B* is transferred to P by two steps, first to the upper excitonic state $P_{Y+}$ with a hundreds of femtoseconds time constant, and then to the lower excitonic state $P_{Y-}$ by internal conversion in about 100 fs. The 240 fs decay in our measurements could be attributed to this energy transfer to P in modified RS601, although we could not distinguish the individual time constants of the two steps from this result. In addition to this fast component, a slower decay with about a 1.6 ps time scale was also obtained from Fig. 2a, but its origin is not clear. It is noted that no apparent bleaching rise was observed at the longer delay time for borohydride-treated RS601. To exclude the influence of so-called photooxidization, the borohydride-treated RS601 was pretreated by sodium ascorbate before the 800 nm pump-probe measurement, and the relevant dynamical trace is shown in Fig. 2b. In addition to the fast bleaching decay, which can also be assigned to the excitation transfer from $B_A^*$ to P, the following bleaching rise in several picoseconds was clearly observed from the transient dynamics. This recovery of the bleaching arises from the contribution of electrochromic shift of the 800 nm band which accompanies the charge separation and electron transfer from P to $H_A$. As to the absence of the bleaching recovery and the presence of a fast decay component of several picoseconds in Fig. 2a for chemically treated RS601, we should firstly consider the influence of photooxidization on the special pair, the energy acceptor. The contribution to the dynamical spectra from accumulation of the special pair cation was suggested to explain a new component with a 400 fs time constant (Jonas et al. 1996). From our results, no 400 fs decay component was obtained from either monoexponential or biexponential fitting of the dynamical trace of chemically modified RS601. On the other hand, the chemical oxidization or photooxidizaton of the special pair would generate a non-zero baseline in time-resolved dynamics, which was supported by our measurements for the chemically oxidized RS601 with potassium ferricyanide and for native RS601 under higher pump intensity (data not given). Furthermore, in our experiments, the normalized transient dynamics of modified RS601 at 800 nm shows no dependence on the pump intensity. Thus, the 1.6 ps component from Fig. 2a would imply the existence of an alternative pathway for energy relaxation in addition to the conventionally accepted channel from B* to P, for example from B* directly to the charge separation state $PB_A^+ H_A^-$ (van Brederode et al. 1999).

The information about charge separation and electron transfer in modified RS601 could be obtained from the direct excitation of the special pair. Figure 3 represents the transient dynamical trace of borohydride-treated RS601 with 865 nm excitation at the magic angle polarization configuration, and the inset shows the time-resolved dynamics in a shorter delay time. By fitting this transient trace, two decay components with 2.8 ps and hundreds of picoseconds time constants were obtained. The 2.8 ps component can be ascribed to the process of charge separation and electron transfer from P to $H_A$,

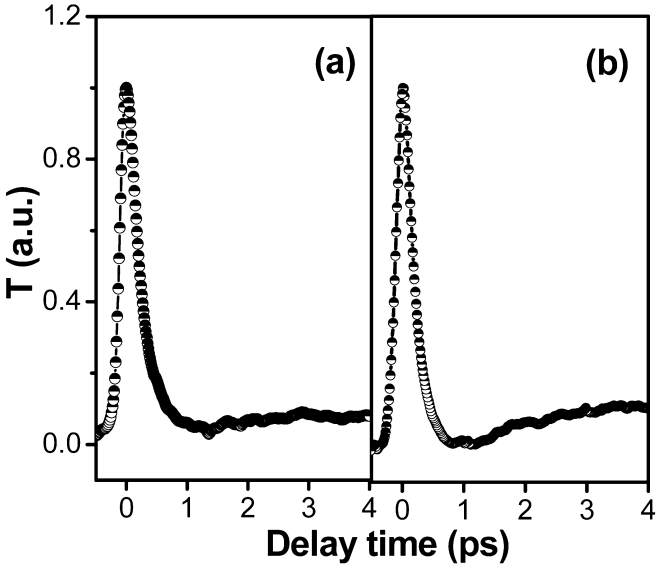


**Fig. 2** Transient transmission change of (**a**) modified RS601 and (**b**) ascorbate-pretreated modified RS601 at 800 nm with parallel pump-probe polarization

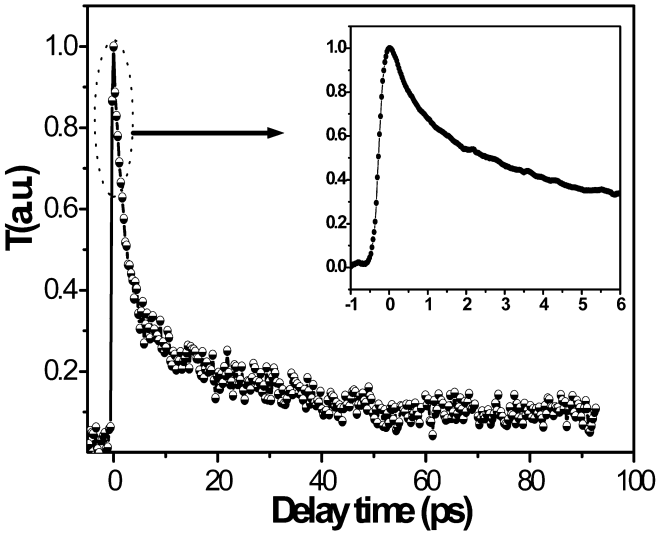


**Fig. 3** Transient dynamical trace of modified RS601 with 865 nm excitation at magic angle (54.7°) pump-probe polarization

which has been confirmed for different RCs of purple bacteria, probably via $B_A^-$ as an intermediate state (van Stokkum et al. 1997). This time constant is in accord with the component of bleaching recovery in Fig. 2b due to the electrochromic shift of the 800 nm band with the presence of the charge separation state $P^+H_A^-$. It is not clear that the existence of $P^+H_A^-$ is a prerequisite for the bleaching recovery at 800 nm. Treatment with sodium borohydride leads to the reduction of $Q_A$, and no electron transfer to $Q_A$ can occur; thus the slower (hundreds of picoseconds) component from Fig. 3 should correspond to the relaxation of the charge separation state by the other channel.

In order to obtain more information about the dynamics of $B_A$ in borohydride-modified RS601, we performed transient measurements with different excitation around the 800 nm band. Figure 4a represents the time-resolved transmission change of modified RS601 at 790 nm, which is similar to that at 800 nm. Fitting the bleaching decay gives two components with lifetimes of 200 fs and 1.4 ps. The ultrafast component could be assigned to excitation transfer from $B_A$ to special P, and the fast one might have the similar origin as that obtained from Fig. 2a. For ascorbate pretreatment of this modified RS601, however, the trace shown in Fig. 4b is obviously different from that at 800 nm excitation: no bleaching recovery was observed at the longer delay time. This dynamical similarity of the modified RS601 with and without sodium ascorbate pretreatment indicates that at least the electrochromic shift has little influence on the absorption band of $B_A$ at 790 nm. On the other hand, the transient dynamics of ascorbate-pretreated modified RS601 shows an ultrafast monoexponential decay with a 170 fs time constant, implying that the alternative channel for energy relaxation, if this channel exists, takes a very small role in excited $B_A$ relaxation. It is known that sodium ascorbate keeps the special pair in a reduced state and will make much more efficient energy transfer and charge separation, and thus might block the excitation transfer through the alternative pathway. In addition, we have no idea about the relationship between the shortening of the fast component for modified RS601 at 790 nm excitation relative to that at 800 nm and the existence of an alternative energy relaxation channel. After all, it can be concluded that the excitation relaxation dynamics at 790 nm mainly originates from the contribution of bacteriochlorophyll in the A-branch and the electrochromic effect primarily affects the red region of the 800 nm band. Meanwhile, in view of the tight packing of pigments and the highly optimized energy transfer and charge separation, it is reasonable to treat the photosynthetic reaction center as a supermolecule. So, it would be not surprising if the alternative energy transfer operates in parallel with the widely accepted excitation transfer channel (van Brederode et al. 1999).

It is commonly accepted that the special pair is a strong interacting bacteriochlorophyll dimer and the excitonic coupling between the two bacteriochlorophylls leads to two distinct bands near 810–825 nm and 870 nm, corresponding to the absorption of the upper excitonic state $P_{Y+}$ and the lower excitionic state $P_{Y-}$, respectively. Evidence has shown that with the direct excitation of the special pair, bleaching could be observed near 810 nm (Reddy et al. 1993; Jonas et al. 1996; Arnett et al. 1999). Figure 5 shows the transient dynamic traces of native (Fig. 5a) and modified (Fig. 5b) RS601 at 818 nm excitation with parallel pump-probe polarization, and Fig. 5c represents the two-color pump-probe result of borohydride-treated RS601 with 800 nm excitation and a 820 nm probe. In

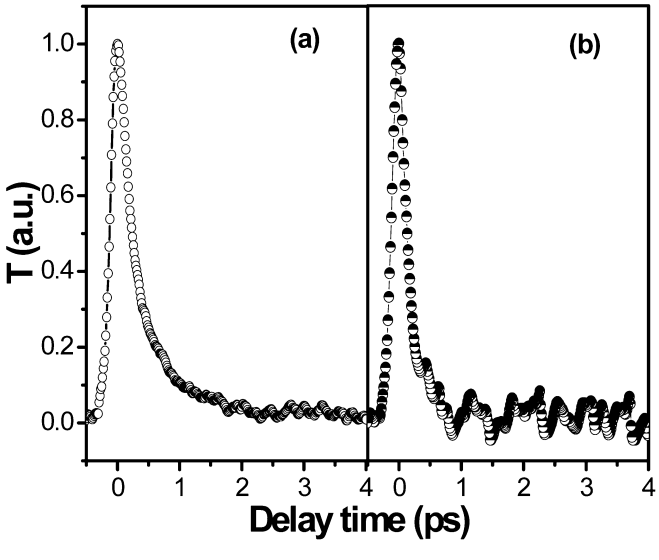


**Fig. 4** Time-resolved dynamics of (**a**) modified RS601 and (**b**) ascorbate-pretreated modified RS601 at 790 nm with parallel pump-probe polarization

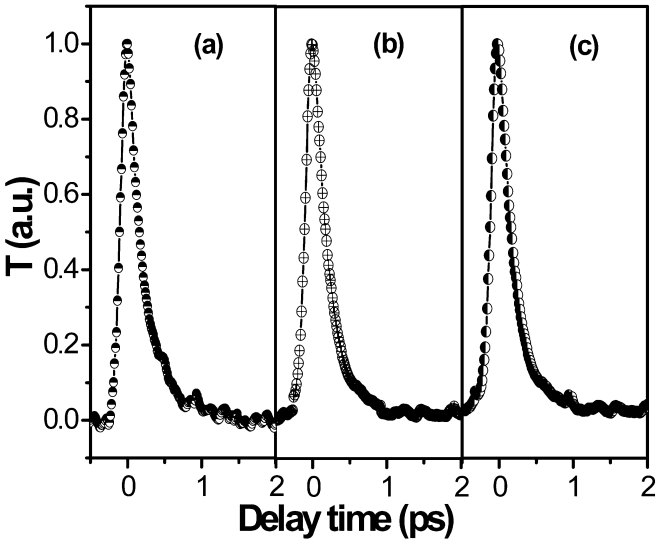


**Fig. 5** One-color pump-probe traces of (**a**) native RS601 and (**b**) borohydride-modified RS601 at 818 nm with parallel polarization, and (**c**) two-color pump-probe dynamics of borohydride-modified RS601 with 800 nm excitation and a 820 nm probe

the first hundreds of femtoseconds, both Fig. 5a and Fig. 5b demonstrate ultrafast bleaching decay with a time constant of about 200 fs, which likely arises from energy transfer from $B^*$ to P and the energy distribution between $P_{Y+}$ and $P_{Y-}$. Considering the concomitant contribution from $P_{Y+}$ and $B_A$, $B_B$ at 818 nm excitation for native RS601, it is difficult from the one-color pump-probe trace to distinguish the individual processes involved. However, Fig. 5c clearly shows that both the establishing of the excited state $P_{Y+}$ and its decay are ultrafast processes with about a 150 fs time scale for modified RS601, which reflects the energy transfer from B to $P_{Y+}$ and the internal conversion from $P_{Y+}$ to $P_{Y-}$, respectively. Thus, these results are in favor of a two-step energy transfer mechanism from B* to P (Jonas et al. 1996; Stanley et al. 1996; Arnett et al. 1999).

In conclusion, we report the ultrafast excitation relaxation dynamics occurring in a modified RS601 reaction center, especially the dynamics of the $B_A$ excited state. The experimental results indicate that the excitation energy at $B_A$ mainly transfers to the higher P excitonic state, and then to the lower state $P_{Y-}$ via internal conversion. The borohydride-modified RS601 still demonstrates photochemical activation, in which the generation of charge separation and subsequent electron transfer mainly affects the $Q_Y$ transition of $B_A$ in the red region. In comparison with the dynamics of the B excited state in native, borohydride-modified and ascorbate-pretreated modified RS610 following selective excitation, an alternative relaxation channel of excitation energy might operate in parallel with the widely accepted energy transfer from B* to the special pair. These results are of significance in understanding the physical mechanism of energy transfer within the photosynthetic reaction center.

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