

## LETTERS

### Observation of Ultrafast Energy Transfer from the Accessory Bacteriochlorophylls to the Special Pair in Photosynthetic Reaction Centers

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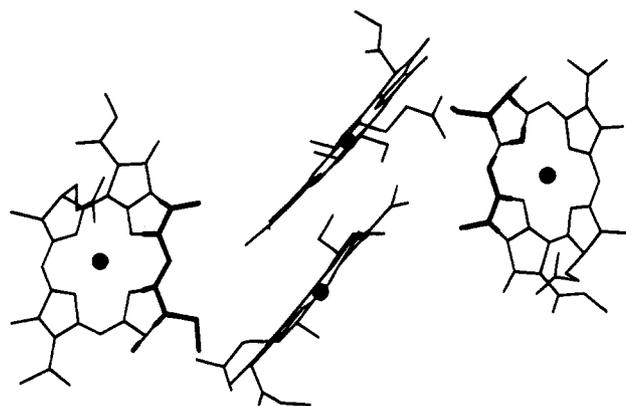
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The energy transfer from accessory bacteriochlorophyll molecules (B) to the special pair (P) in the reaction center of *Rb. capsulatus* and *Rb. sphaeroides* R26 has been time-resolved using 23 fs pulses. In both reaction centers, the B\* transient bleaching decays biexponentially, with a fast component of approximately 100 fs which gives a characteristic time scale for excitation energy transfer from B\* to P. In *Rb. capsulatus*, the amplitudes for this component and a 360 ± 40 fs decay of uncertain origin reported in previous experiments are approximately equal. Several models for the two components are discussed.

#### Introduction

In photosynthesis, light energy is captured by antenna molecules and efficiently transferred to a special pair of bacteriochlorophylls (BChl) located in a reaction center (RC) where the primary electron transfer, which is used to drive biochemical reactions, occurs.<sup>1</sup> The RCs from purple photosynthetic bacteria contain six pigments involved in initial charge separation: two BChl in the special pair (P), two accessory BChls (B), and two bacteriopheophytins (H).<sup>2</sup> These six pigments are anchored to a protein scaffold along two branches (which meet at the special pair) with an overall approximate C<sub>2</sub> symmetry (see Figure 1). The primary electron transfer from P to H, which are separated by 17 Å center to center, takes place only along one branch in about 3 ps, 1000 times faster than expected for electron transfer across a vacuum. There has been a great deal of controversy about whether the accessory BChls, which lie between P and H, act as real<sup>3</sup> and/or virtual intermediates in the primary electron transfer (see references in ref 4) and also about the extent of excitonic coupling between the six pigments.<sup>5-8</sup> In isolated reaction centers, the energy transfer from electronically excited accessory BChls to the special pair is also known to be orders of magnitude faster than



**Figure 1.** Partial structure of the reaction center of *Rb. sphaeroides* (the reaction center of *Rb. capsulatus* wild type is similar) showing the proximity between the accessory bacteriochlorophylls (B) and the special pair (P). The view is from the top with the approximate C<sub>2</sub> axis of the reaction center pointing out of the plane of the figure: the two molecules in the center are the special pair, and the other two molecules are the accessory bacteriochlorophylls. Bonds between accessory bacteriochlorophyll nuclei which lie within 5 Å of the special pair are shown as thick lines.

predicted by a naive application of weak coupling Förster energy transfer theory,<sup>9</sup> and a careful investigation of energy transfer may shed light on the coupling between pigments in the reaction

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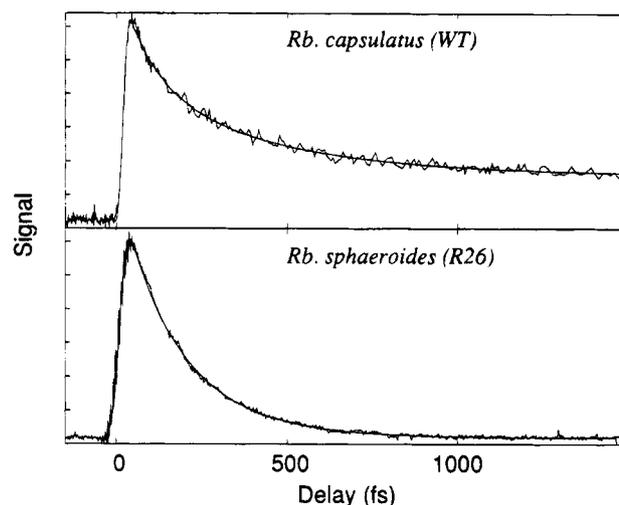
center. One important difference between energy transfer and electron transfer is that energy transfer is sensitive to the HOMO–LUMO energy gap on one chromophore compared to the HOMO–LUMO energy gap on another chromophore, which is available spectroscopically, while electron transfer is sensitive to the relative energies of orbitals on different chromophores (donor's HOMO and acceptor's LUMO). It has been known for some time that excitation of B gives a quantum yield of 0.93 for electron transfer,<sup>10</sup> compared to a quantum yield of 1 for excitation of P. Previous work has not time-resolved either the decay of B\* or the rise of P\* during the ultrafast energy transfer process from B to P, but several different experiments have shown that both take place in less than 100 fs,<sup>11,12</sup> and one recent experiment<sup>13</sup> yielded 200 fs.

Recent measurements of the time-resolved room temperature and low temperature<sup>14–16</sup> fluorescence with high signal to noise ratio have shown that the decay of the primary electron donor state (P\*) is nonexponential. Oscillations, which had been previously observed in pump–probe experiments,<sup>17–19</sup> are most likely due to vibrational quantum beats and originate from P\*. Consequently, the primary electron transfer event occurs under vibrationally unrelaxed conditions. In addition to the rise and decay of transient bleaching and excited state absorption of various bands, electrochromic shift and vibrational relaxation may also cause some bands of the photoexcited reaction center to continuously evolve in time (i.e., shift frequency). Spectral evolution after direct excitation of P has been recorded with 45 fs pulses<sup>6</sup> and attributed to a relaxation process within the excited state of P. The time scale of the relaxation was estimated to be 90 fs to a few hundred femtoseconds and is comparable to the time scale of the energy transfer. In other words, the energy transfer process is also likely to occur under vibrationally unrelaxed conditions and may be nonexponential. In this Letter, we report measurements of this energy transfer step by monitoring the transient absorption of B.

## Experimental Section

**Materials.** Reaction centers of *Rb. capsulatus* wild type and *Rb. sphaeroides* R26 were isolated and purified as described previously.<sup>20,21</sup> The absorbance of the samples at 800 nm was 0.026 (*capsulatus*) and 0.051 (R26) in a 100  $\mu\text{m}$  optical path length. Samples with and without sodium ascorbate (33 mM) were used. Sodium ascorbate reduces the special pair cation in photooxidized RCs, leaving a reduced quinone which blocks secondary electron transfer, causing primary charge recombination on a 10 ns time scale.<sup>1</sup> (A fraction of the charge recombination products are produced in a triplet state with 6  $\mu\text{s}$  lifetime.) Measurements were also carried out on an R26 sample in which the special pair was chemically oxidized by 100 mM potassium ferricyanide.<sup>22</sup> The stability of our laser system makes measurements at such low optical density possible. The sample was circulated by a peristaltic pump with a speed of  $\sim 6$  cm/s (80 cm/s for R26) in the 100  $\mu\text{m}$  quartz flow cell. The total volume of 1.8 mL was cooled by ice water.

**Laser System.** The Kerr lens mode-locked Ti:sapphire laser is home-built according to the design of Asaki et al.<sup>23</sup> The laser is pumped by 6 W all lines of Ar<sup>+</sup> laser as described elsewhere.<sup>24</sup> A Bragg cell was added to the laser to allow cavity dumping at up to  $\sim 1$  MHz. For this experiment, it was necessary to lengthen the pulse duration in order to narrow the frequency spectrum and minimize electronic excitation of P: the frequency spectrum was nearly Gaussian with a fwhm bandwidth of 45 nm (38 nm for R26) centered at 798 nm (803 nm for R26). The autocorrelation function taken with a 100  $\mu\text{m}$  BBO (type I) had a 33 fs fwhm (38 fs for R26). A fit assuming a Gaussian pulse



**Figure 2.** Transient change in absorption of reaction centers from wild type *Rb. capsulatus* (top panel) and the R26 mutant strain of *Rb. sphaeroides* (bottom panel) as a function of pump–probe delay. Transient absorption was recorded with  $\sim 25$  fs pump and probe pulses centered at  $\sim 800$  nm, which excites the accessory bacteriochlorophylls in the reaction center. A positive signal represents a decrease in absorbance. In both cases, the early part of the decay can be fit as biexponential with roughly equal amplitude for exponential lifetimes of about  $\sim 100$  and  $\sim 360$  fs. Note the difference in asymptotic base line for the two reaction centers on this time scale. The  $\sim 100$  fs decay represents a characteristic time scale for ultrafast energy transfer from the accessory bacteriochlorophylls (B) to the special pair (P). Quantum beat oscillations appear in the *Rb. sphaeroides* (R26) signal, which also has interferometric oscillations at the 2.6 fs optical period on the rising edge. In *Rb. sphaeroides*, pump–probe data covering 20 ps delay require an additional 2.8 ps exponential rise.

shape gave a 23 fs pulse duration (27 fs for R26), near the transform limit. The experimental setup is a general pump–probe configuration with parallel pump and probe polarizations. A double pass through a pair of LaKL21 prisms precompensates for material dispersion. The probe pulse was a first surface s-reflection from a BK7 window ( $\sim 5\%$ ) which passed through a second BK7 window so that the dispersion of the pump and probe beam paths was the same. The repetition rate and pump–probe energy used in the experiment were 152 kHz and 1.5 nJ, respectively. Preliminary experiments using 0.75 and 1.5 nJ pulse energies did not perceptibly differ from each other. The spot size in the sample was about 70  $\mu\text{m}$ . The probe beam was monitored by a red-sensitive photodiode and lock-in detection referenced to the  $\sim 2$  kHz frequency of an optical chopper in the pump beam path. Analysis of the measured kinetics and fitting of the curves were carried out essentially as before<sup>4</sup> and modified to fit variable time steps.

## Results and Discussion

Figure 2 shows the transient absorption signal for the sodium ascorbate reduced sample of reaction centers of *Rb. Capsulatus* (top) and an untreated R26 sample (bottom). The signal includes positive contributions from both the recovery of ground state absorption and excited state stimulated emission, but may also include negative contributions from absorption by the excited state. The differences at long time may reflect both the different absorption spectra of the two RCs and the different pulse spectra. Quantum beat oscillations are present in the R26 signal. The signals apparently lack the “coherence spike” which is usually observed at zero delay in pump–probe experiments. In a simple two-level resonance model,<sup>25,26</sup> the magnitude of the coherence spike depends on the ratio of the pulse duration,  $\Delta t$ , to the electronic dephasing time,  $T_2$ . For  $\Delta t/T_2 \sim 1/5$ , which is plausible for our experiment, computer simulations<sup>25,26</sup> have

shown that the coherence spike is very small relative to the signal if both pump and probe are on resonance. A satisfactory fit ( $\chi^2 = 1.16$ ) to our entire transient absorption scan for the *capsulatus* sample with sodium ascorbate reducing agent can be obtained by convoluting three exponentials with the instrument response:  $A_1 = 42 \pm 10\%$ ,  $\tau_1 = 65^{+35}_{-15}$  fs;  $A_2 = 40 \pm 10\%$ ,  $\tau_2 = 360 \pm 40$  fs; and  $A_3 = 18\%$ ,  $\tau_3 = 22$  ps. Similar constants were obtained from a fit which began 40 fs after time zero, demonstrating that the effect of any residual coherence spike on the fit parameters is negligible. Starting 50 fs after time zero, the best fit for *R26* is  $A_1 = 62 \pm 15\%$ ,  $\tau_1 = 116 \pm 35$ ;  $A_2 = 40 \pm 15\%$ ,  $\tau_2 = 270^{+90}_{-40}$ ;  $A_3 = -6\%$ ,  $\tau_3 = 2.8$  ps; and  $A_4 = 4\%$ ,  $\tau_4 = \infty$ . It must be emphasized that the two fast decays have similar time constants which are highly correlated both to each other and to their amplitudes in both RCs.<sup>27</sup> Although one might expect the energy transfer rates to differ in the two RCs because the spectral overlap between B and the lower exciton component of P is different, we have not yet established a discrepancy between microscopic energy transfer time scales in the two reaction centers. The slowest decaying component ( $\tau_3$ ) in *Rb. capsulatus* is not well determined in our experiments. For *Rb. capsulatus*, the best fit to signals from the sample without sodium ascorbate gave slightly different time constants and amplitudes for the  $\tau_1$  and  $\tau_2$  components, but we were unable to establish a significant discrepancy. Addition of ascorbate had no effect on the higher signal-to-noise *R26* signal, which was flowed more rapidly. Addition of ferricyanide to *R26*, which oxidizes P and eliminates the 870 nm absorption band of P, increases the amplitude of  $A_1$  relative to  $A_2$  and eliminates the 2.8 ps rise time. The  $\sim 100$  fs component is the fastest energy transfer ever resolved in these systems, and its relatively low amplitude ( $\sim 50\%$ ) may have made it difficult to detect.

The  $\sim 100$  fs transient resolved here seems to be associated with energy transfer from  $B^*$  to P. This assignment is consistent with estimates from prior work.<sup>11,12</sup> With 100 fs time resolution, Breton et al.<sup>11</sup> have reported an instrument limited rise of P—upon excitation of B or H and estimated that the energy transfer from  $B^*$  to P is faster than 100 fs. One recent report<sup>13</sup> assigns a 200 fs decay, observed at 1215 nm following 800 nm excitation, as  $B^*$  to P energy transfer. Stanley and Boxer<sup>14</sup> state that a rise time is required to fit the rise of  $P^*$  emission at 955 nm observed by fluorescence up-conversion following excitation of B at 803 nm. From a room temperature study of *R26* RCs in which B was excited with 120 fs pulses with 10 nm bandwidth centered at 800 nm, Bradforth, Jimenez, and Fleming (unpublished results) obtain a best fit rise time of 120 fs for up-converted  $P^*$  fluorescence at 930 nm. The  $250\text{ cm}^{-1}$  width of the B band at 4 K sets a lower limit of 20 fs for the lifetime of  $B^*$ , and Small and co-workers have estimated a lower bound of 30 fs from hole-burning studies.<sup>5</sup> Comparing the  $500\text{ cm}^{-1}$  absorption bandwidth of B at room temperature to a 50 fs lifetime broadened line width of  $100\text{ cm}^{-1}$ , it seems that at room temperature the band is significantly inhomogeneously broadened. It also seems likely that energy transfer from  $B^*$  to P is reversible at this time scale and competes with relaxation processes in the special pair.

The 360 fs component ( $\tau_2$ ) has been temporally resolved in prior studies ( $\sim 400$  fs),<sup>11,12</sup> but the origin of this component is uncertain. The 400 fs time scale appears as either the decay time of a transient bleach or the rise time of a transient increase in absorbance when either B or H is excited, but does not appear when P is excited directly<sup>12</sup> and does not appear in the rise of  $P^*$  fluorescence when B is excited. Thus, the 400 fs component must be associated with excitation of B. Several explanations for the 400 fs component have been proposed: (1) The 400 fs

component is the decay time for the transient bleaching of a small fraction (about 10%) of the reaction centers in a state (suggested to be conformationally constrained)<sup>12</sup> for which  $B^*$  decays to a state other than  $P^*$ . In other words, a small portion of reaction centers is incapable of transferring energy to P. (2)  $B^*$  not only transfers energy to P but also decays to an intermediate which subsequently relaxes to ground state B on a 400 fs time scale.<sup>11</sup> For this scheme, the excited state contribution to the pump—probe signal decays at the sum of the rates for the two parallel excited state decay processes while the ground state bleach is filled in by both energy transfer ( $\sim 100$  fs) and also by the 400 fs decay of the intermediate. (3) The 400 fs component is due to the absorption of a second photon from the pump pulse, forming  $B^*P^*$ . This suggestion is consistent with the observation that the 400 fs component is power dependent with microjoule pulse energies.<sup>11</sup> Assuming that electron transfer follows energy transfer from  $B^*$  to P, models 1 and 2 require an  $A_1(100\text{ fs}):A_2(400\text{ fs})$  amplitude ratio of 9:1 in order to reproduce the observed electron transfer quantum yield of 0.93 and are ruled out by the approximately equal amplitudes observed in our experiment. Model 3 is inconsistent with our low pulse energy (1.5 nJ): the amplitude of the 400 fs component relative to the  $\sim 100$  fs component is too large for a doubly excited state. (We calculate that each pulse excites only 5% of the reaction centers in the focal volume to  $B^*$ .) Other models which have been ruled out previously will not be discussed here.<sup>12</sup> Strongly bimodal decays of the initially excited state are observed in Redfield calculations<sup>28,29</sup> when vibrational relaxation occurs on a similar time scale to that set by the electronic coupling, but this cannot explain the absence of a 400 fs rise in  $P^*$ . Because no time scale of 400 fs or longer appears in the rise of  $P^*$ , the 400 fs component cannot represent processes which take place before energy transfer from  $B^*$  to P: one possibility is that the 400 fs component is related to relaxation processes within either  $P^*$  or the ground state of B after energy transfer from  $B^*$  to P.

In the *R26* sample, we assign the 2.8 ps rise time to the electrochromic band shift of B caused by formation of the electron transfer product  $P^+H^-$ .<sup>12</sup> In *Rb. capsulatus*, the long lifetime component ( $\tau_3$ ) appears as a slow decay instead of the 2.8 ps rise time associated with  $P^+H^-$  formation, possibly because the excitation and detection wavelength (800 nm) is close to an apparent isosbestic point of the 800 nm band shift.<sup>12</sup> The 2.8 ps rise does not occur in oxidized RCs where no electron transfer occurs.

Although there seems to be an experimental and theoretical consensus that the special pair is a strongly excitonically coupled dimer and that the two bacteriopheophytins function essentially as monomers, there has been some controversy about the degree of excitonic coupling between the two B molecules.<sup>5,6</sup> The hypothesis that the two B molecules function as monomers and that the two time scales observed in our experiment reflect separate time scales between the two branches of the reaction center is not compatible with experiments which selectively excite one of the bacteriopheophytins (transitions to  $H_L$  and  $H_M$  can be spectrally resolved at low temperature): both selective excitations yield a 400 fs decay, implying that the 400 fs component is not associated with only one branch of the reaction center.<sup>12</sup> The hypothesis that the two components arise from the excitonically coupled states  $B_+$  and  $B_-$  leads one to expect two energy transfer rates because the coupling of these two states to  $P_+$  and  $P_-$  may differ. However, excitonically coupled models for the reaction center, such as the Won—Friesner Hamiltonian,<sup>8</sup> predict that  $B_-$  dominates the pump—probe signal by about 14:1 because the transition dipole is larger,<sup>5</sup> so that

only one energy transfer time would be recovered unless the signal to noise was very high.

Many challenging questions arise from our experimental result. For example, (1) Is the energy initially transferred to the upper exciton component of the special pair ( $P_+$ )?<sup>9</sup> If so, then a subsequent energy transfer step from  $P_+$  to  $P_-$  must also be complete within about 120 fs. Direct energy transfer from B to  $P_-$  seems incompatible with the appearance of the same time scales in oxidized reaction centers where the  $P_-$  state does not exist. However, it is known that the special pair cation has an absorption band near 795 nm, which is close to that of the upper exciton component in the normal RC (810 nm).<sup>1,30</sup> (2) What is the  $\sim 100$  fs process in the oxidized reaction center? (3) Why do we have approximately equal amplitudes for the  $\sim 100$  and 400 fs components? (4) Does all the electron transfer observed after excitation of  $B^*$  proceed through the lower exciton component of the special pair  $P_-$ ? (5) How does vibrational excitation or coherence affect the energy transfer rate?<sup>28,29</sup> (6) Could the hypothesis of hole transfer involving  $B^{31,32}$  be relevant to our results? If the HOMO of excitonically coupled B lies below the HOMO of P and the LUMO of B lies above the LUMO of H, then stepwise electron-hole transfer may be possible from  $B^*$ . (Electron transfer from  $P^*$  is different because it depends on the relative energy of the LUMOs.)

### Conclusions

The energy transfer from B to P has been time-resolved and is ultrafast ( $\sim 100$  fs). The amplitude for the short energy transfer component in the decay of  $B^*$  is about equal to that for the  $\sim 400$ -fs decay resolved in prior studies. If electron transfer occurs only from  $P_-$ , this rules out some previously suggested hypotheses about the origin of the 400 fs component. Energy transfer occurs on a time scale similar to that for vibrational relaxation. The mechanism of this ultrafast energy transfer is unclear, but is apparently not simply Förster energy transfer, and probably involves other short range interactions.<sup>33</sup>

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