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Excited-state energy transfer pathways in photosynthetic reaction centers: 5. Oxidized and triplet excited special pairs as energy acceptors

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Abstract

In bacterial photosynthetic reaction centers, ultrafast singlet excited-state energy transfer occurs from the monomeric bacteriochlorophylls, B, and bacteriopheophytins, H, to the homodimer special pair, P, a pair of strongly interacting bacteriochlorophylls. Using fluorescence upconversion spectroscopy, energy transfer to the special pair can be monitored by observing the decay of ^{1}B emission and/or the rise of ^{1}P. We report ^{1}B decay kinetics following excitation in the H band in reaction centers where the homodimer and heterodimer (M202HL) special pairs are oxidized, P^+ and D^+, respectively, and when the homodimer special pair is in the triplet state, ^{3}P. In wild type and the M71GL mutant (a carotenoid-less reaction center), the rates of ^{1}B decay when P^+ and ^{3}P are present, $(\sim 260\ \text{fs})^{-1}$ and $(\sim 235\ \text{fs})^{-1}$, respectively, are similar to that for energy transfer to ^{1}P $(\sim 190\ \text{fs})^{-1}$ in wild type measured by either the fluorescence decay of ^{1}B or the rise of ^{1}P. In contrast to the homodimer special pair in wild type where the energy transfer rates along the two branches are very similar, singlet energy transfer from the monomeric chromophores along the L and M branches to the heterodimer special pair is asymmetric and is slower along the L side. The ^{1}B decay in wild type is well described by a single rate constant of $(\sim 190\ \text{fs})^{-1}$ and in M202HL exhibits two components with rate constants $(\sim 780\ \text{fs})^{-1}$ and $(\sim 250\ \text{fs})^{-1}$. In M202HL reaction centers containing D^+, ^{1}B decays with a single rate constant of $(\sim 343\ \text{fs})^{-1}$; hence, the energy transfer rates along the two branches become similar. Thus, while conversion of the special pair homodimer to a heterodimer breaks the symmetry of ultrafast energy transfer along the two branches of chromophores, symmetry can be restored by oxidizing the heterodimer special pair. To our knowledge, this is the first report of such dramatic alteration of energy transfer within a single reaction center protein. These findings bear on the mechanism of energy transfer in the reaction center and may provide insight into the differences in the electronic interactions on the L vs. M sides of the RC that are relevant to unidirectional *electron* transfer.

1. Introduction

The bacterial photosynthetic reaction center (RC) is responsible for the initial light-driven charge separation events in photosynthesis. Light energy absorbed by antenna complexes is funneled

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to the special pair (P) in the RC; ^{1}P then transfers an electron to an electron acceptor, rapidly trapping the excitation energy in a transient charge-separated species. A schematic diagram based on the X-ray structure [1] illustrating the arrangement of the relevant chromophores is shown in Fig. 1. In isolated RCs, excitation of the special pair can be achieved by rapid and efficient singlet excited-state energy transfer from the monomeric bacteriopheophytins and bacteriochlorophylls. The chromophores labeled B_L and B_M are monomeric bacteriochlorophylls on the functional and non-functional sides, respectively, of the RC; the chromophores labeled H_L and H_M are monomeric bacteriopheophytins on the functional and non-functional sides, respectively. Functional is used here to denote the electron transfer process $^1P \rightarrow P^+H_L^-$ that is found to occur almost exclusively in normal RCs at all temperatures, despite the structural symmetry of the RC that suggests $^1P \rightarrow P^+H_M^-$ might be equally likely to occur.

The rate of singlet excited-state energy transfer from the B and H chromophores to P can be probed by femtosecond transient absorption spectroscopy [2–6] or by measuring the rise of ^{1}P fluorescence using fluorescence upconversion. In previous work on wild type (WT) and the M214LH (the β mutant, where a bacteriochlorophyll, β_L, replaces H_L) and M182HL (the θ mutant, where a bacteriopheophytin, θ_M, replaces B_M) mutants, we showed that the rates of energy transfer along the L and M branches of chromophores are comparable [7,8]. Specifically, ^1B_M and ^1B_L transfer energy to P in about 160 fs. We then showed that the rate of singlet energy transfer from the excited B and H chromophores to P can also be probed by measuring the rise and decay of ^{1}B emission after excitation of the B or H chromophores using fluorescence upconversion. In wild-type, M182HL, and M202HL RCs, the excited-state decay of ^{1}B closely matches the rise of fluorescence from ^{1}P (or ^{1}D), and following excitation of H, energy transfer occurs by a 2-step sequential mechanism: $^1H \rightarrow B \rightarrow P$ (or D) [8].

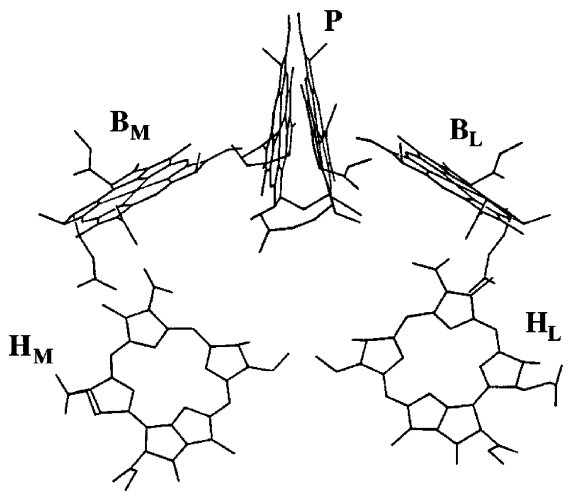

Fig. 1. Schematic diagram of the chromophores involved in the energy and electron transfer processes of isolated wild-type *Rb. sphaeroides* photosynthetic reaction centers taken from the X-ray structure.

In the M202HL heterodimer mutant, one of the coordinating histidine ligands to the special pair, histidine M202, is replaced by leucine and the central Mg^{2+} ion is lost from the bacteriochlorophyll, resulting in the incorporation of a bacteriopheophytin [9]. The Q_Y absorption band of the special pair (designated D in the heterodimer mutant) is dramatically perturbed with respect to wild type as shown in Fig. 2. It appears as a much broader absorption with poorly resolved features at 840 and 920 nm at 77 K [10,11]. Electron transfer in the heterodimer mutant remains unidirectional; however, the excited-state dynamics of ^{1}D are substantially different from wild type [9,12–16]. By monitoring the decay of emission from ^{1}B at 815 nm or the rise of emission from ^{1}D at 1040 nm, we found that energy transfer along the L side is substantially slower than along the M side [17,18]. Specifically, it was found that $^1B_L \rightarrow D$ occurs in ~700 fs, while $^1B_M \rightarrow D$ occurs in ~190 fs [18]. We denote this *asymmetric energy transfer*, in contrast with what is observed in homodimer-containing RCs [19].

The M182HL mutant proved to be useful in these studies because the RC assembles with a bacteriopheophytin in place of the normal bacteriochlorophyll in the B_M binding site [20], enhancing the resolution of features around 800 nm and making selective excitation of the chromophores in the B_M and B_L binding sites possible [18]. In a separate line of investigation we also found

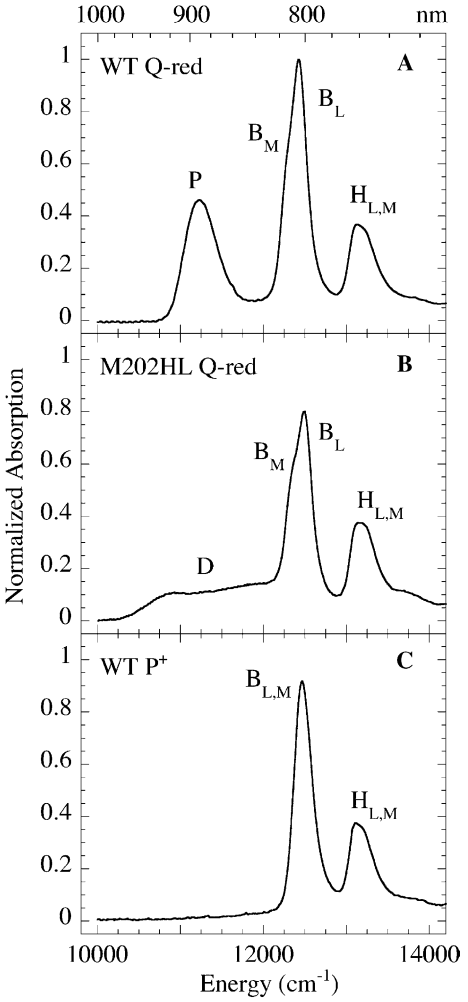

Fig. 2. Absorption spectra in the Q_Y region at 77 K for *Rb. sphaeroides* WT Q_A-reduced RCs (A), M202HL Q_A-reduced RCs (B) and WT P-oxidized RCs (C), normalized to their maximum intensity in the H-band region. The spectra are plotted in linear energy units; units in nanometers are indicated along the upper horizontal axis.

that replacement of bacteriopheophytin in the B_M binding site raises the activation barrier for triplet energy transfer: $^3P \rightarrow B_M \rightarrow$ carotenoid [21]. In this mutant, a substantial fraction of the RCs could accumulate in the ^3P state during high repetition rate excitation, in contrast to wild type where triplet energy transfer to the carotenoid is very rapid and a negligible population is found in the ^3P state (see discussion). Nonetheless, the decay of ^1B_L and rise of ^1P were unaffected in M182HL, leading us to probe singlet excited-state dynamics when the circumstances are arranged to intentionally place the special pair in the ^3P or oxidized state. As far as we know, emission has never been observed from the *excited* states of P^+ or ^3P, thus fluorescence upconversion studies are limited to probing the dynamics of ^1B. In the following, we report ^1B decay dynamics in P^+, D^+, and ^3P reaction centers. In all cases, ^1B decays on a time scale comparable with what is observed when the special pair is in the neutral ground state. Subtle variations, presumably reflecting the difference in electronic structure of the acceptor, are observed.

2. Experimental

2.1. Sample preparation

The codons for the M202HL and M71GL mutations were inserted into the poly-His wild-type background. Wild-type, M202HL, and M71GL *Rb. sphaeroides* were grown semi-aerobically and isolated rapidly by the procedure designed to take advantage of the poly-His tag engineered into the RCs [22]. RCs were suspended in 10 mM Tris–HCl (pH 8.0), 0.1% Triton X-100 (0.1% LDAO for Q_A-depleted RCs), and 1.0 mM EDTA. For experiments involving Q_A-depleted RCs, removal of Q_A was achieved according to the procedure of Okamura et al. [23] For experiments in which Q_A was pre-reduced, sodium dithionite was added to a final concentration of 5 mM just prior to the measurement. For experiments involving the chemically oxidized special pair, potassium ferricyanide was added to a final concentration of 500 mM. All samples were dissolved in 1/1 (v/v) glycerol/buffer solution. The RCs were concentrated in order to achieve a sufficient optical density in the 75 μm path length cell, typically 0.1–0.2 at 800 nm. Fig. 2 shows the Q_Y absorption region of WT Q_A-reduced RCs, M202HL Q_A-reduced RCs, and chemically oxidized P^+ RCs [24].

2.2. Fluorescence upconversion spectroscopy

The low-temperature fluorescence upconversion spectrometer has been described in detail

previously [7,25]. Briefly, samples were excited using a mode-locked Ti:sapphire laser (Spectra Physics Tsunami) pumped by 6–10 W (all lines) from an argon-ion laser (Spectra Physics Model 2080). For experiments exciting at 760 nm in the H band, the pulse widths were ~110 fs with a time-bandwidth product typically less than 0.41. Samples were excited with magic angle polarization relative to the gate beam with ~15 mW of 760 nm light at 82 MHz (<200 pJ of energy per pulse) focused down to a ~50 μm diameter spot size. Low temperature (85 K) was achieved using a miniature Joule-Thompson refrigerator (MMR Technologies, Mountain View, CA) and very thin sample geometry. The sample is rastered between scans, typically every few minutes, to prevent sample degradation in the excitation beam. For experiments where the decay of ^{1}B is compared between two samples, e.g., Q_A-reduced vs. Q_A-containing wild-type RCs, the samples (of comparable concentration) were loaded into separate compartments in the refrigerator so that the kinetics could be probed under identical conditions by translation of the sample. This proved to be a very accurate method for making comparisons. The individual data sets were collected with a delay line step size of 21 fs/point over the first 7.5 ps of the decay.

2.3. Data analysis

The data were fit to the convolution of the instrument response function with a model function composed of a sum exponentials, a baseline and a time offset. All data sets required a rise component reflecting the $^1H \rightarrow B$ energy transfer rate to obtain a good fit to the data. Overlaying the data sets (Figs. 4–6) revealed clear differences in the decay of ^{1}B emission but no discernable differences in the rise of ^{1}B emission. To determine the decay lifetimes of ^{1}B emission, the rise time reflecting $^1H \rightarrow B$ energy transfer was fixed to 100 fs, which was the average rate found by fitting the rise times across all data sets ($\tau_{rise} = 99 \pm 31$ fs, $n = 64$ data sets). Fixing the rise lifetime generally resulted in smaller errors for the values of the ^{1}B emission decay lifetimes [26]. The fits to the data generally yielded reduced χ^2 of less than 1.3 with unstructured residuals. The values reported are averages of the fit values from three or more data sets and the errors are the standard deviation of the averages.

3. Results

3.1. Strategies for preparing samples with special pairs in different states

Fig. 3 illustrates the reaction scheme used simulate the RC steady-state populations under experimental conditions and the resulting RC state is listed in Tables 1 and 2 for each RC sample. The average power delivered to the samples is 15 mW; however, the individual pulses are very weak (<200 pJ), so that the fraction of the sample excited with each pulse is <2%. For most experiments reported in parts 1–4 of this series, RCs were either depleted of Q_A or Q_A was pre-reduced with sodium dithionite. If this is not done, then it is not possible to observe the emission from ^{1}P

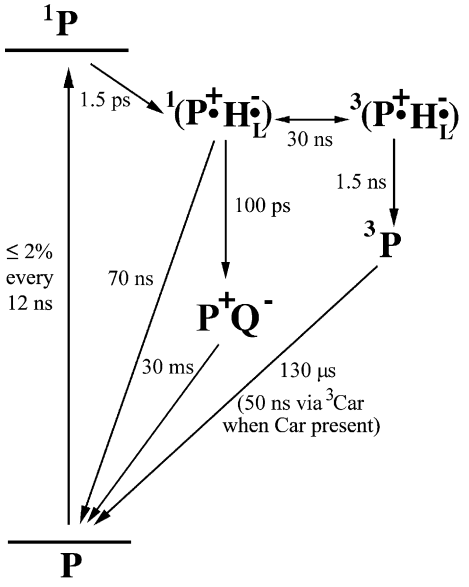

Fig. 3. Reaction scheme illustrating the relevant states and rates of the RC at 85 K used to model the steady-state populations of the sample under experimental conditions (see text for details).

Table 1
Time constants (fs) and amplitudes (%) for the decay of spontaneous fluorescence from ^{1}B detected at 815 nm in wild-type and M71GL RCs under different conditions at 85 K for excitation of the H band at 760 nm

RC sample	RC state	815 nm emission decay components	
Wild type	P Q_A^-		
Q_A-reduced	A (%)	99.4 ± 0.2	0.6 ± 0.2
	τ (fs)	189 ± 6	Several ps
Wild type	P^+ Q_A^-		
	A (%)	99.8 ± 0.1	0.2 ± 0.1
	τ (fs)	261 ± 4	Several ps
Wild type	P^+ Q_A^-		
+ menadione	A (%)	99.4 ± 0.2	0.6 ± 0.2
	τ (fs)	252 ± 5	Several ps
Wild type	P^+ Q_A		
P-oxidized	A (%)	99.7 ± 0.1	0.3 ± 0.1
	τ (fs)	271 ± 3	Several ps
Wild type	P		
Q_A-depleted	A (%)	95.2 ± 0.5	4.8 ± 0.5
	τ (fs)	225 ± 8	Several ps
M71GL	^{3}P		
Q_A-depleted	A (%)	96.8 ± 0.5	3.2 ± 0.5
	τ (fs)	236 ± 19	Several ps

"Wild type" is an RC with Q_A, carotenoid, and P in its neutral ground state. In Q_A-reduced RCs, Q_A was chemically reduced with sodium dithionite prior to the experiment; Q_A-depleted RCs lack Q_A. In P-oxidized RCs, the RCs were treated with potassium ferricyanide prior to the experiment; and "+ menadione" refers to samples treated with menadione prior to the experiment to insure full occupancy of qunione in the Q_A binding site (see text).

since the entire sample is in the $P^+Q_A^-$ state at steady state ($P^+Q_A^-$ decays in about 30 ms at 85 K) [27]. However, we can exploit this situation and prepare RCs in the $P^+Q_A^-$ state by illumination, as an alternative to chemical oxidation of P using ferricyanide, and still measure ^{1}B dynamics. Especially in the case of the M202HL heterodimer, whose oxidation potential is much greater than the wild-type special pair, very strong oxidizing agents are required to form D^+ [12,15], therefore steady-state formation of $D^+Q_A^-$ in the excitation beam is the preferred method for studying D^+-containing RCs.

With electron transfer from H_L^- to Q_A blocked by Q_A pre-reduction or removal, $P^+H_L^-$ recombination returns the RC either to the ground state or the triplet excited state of the special pair, ^{3}P, with the yield of the latter approaching unity at cryogenic temperatures [28,29]. Carotenoid was present in wild type and the mutant strains we have studied, and the carotenoid quenches ^{3}P in about 50 ns [30] and then decays non-radiatively to the ground state. Thus, in carotenoid-containing RCs, the special pair recovers to the ground state (from both ^{3}P and $^1(P^+H_L^-)$) in ~65 ns and at most 4% of RCs are expected to be in the ^{3}P state at steady state under the conditions of our experiments.

It was recently reported that replacement of the glycine residue at position M71, which is located near one end of the carotenoid binding pocket, with a bulky leucine leads to RC assembly without

Table 2
Time constants (fs) and amplitudes (%) for the decay of spontaneous fluorescence from ^{1}B detected at 815 nm in M202HL heterodimer RCs under different conditions at 85 K for excitation of the H band at ~760 nm (see Table 1 for sample descriptions)

RC sample	RC state	815 nm emission decay components		
M202HL	D Q_A^-			
Q_A-reduced	A (%)	67.0 ± 1.8	32.6 ± 1.8	0.4 ± 0.2
	τ (fs)	295 ± 38	817 ± 40	Several ps
M202HL	D^+ Q_A^-			
	A (%)	98.9 ± 0.3	1.1 ± 0.3	
	τ (fs)	348 ± 7	Several ps	
M202HL	D^+ Q_A^-			
+ menadione	A (%)	97.8 ± 0.1	2.2 ± 0.2	
	τ (fs)	354 ± 8	Several ps	
M202HL	D			
Q_A-depleted	A (%)	73.3 ± 2.5	25.4 ± 2.4	1.1 ± 0.1
	τ (fs)	278 ± 27	815 ± 48	Several ps

carotenoid [31]. The M71GL RC has the same phenotype as RCs extracted from the carotenoid-less R26 strain, and we have found that their triplet yield and dynamics are essentially identical with R26 RCs [32]. In the absence of carotenoid, ^{3}P decays in approximately 130 μs at 85 K, consequently ~99% of the sample is predicted to be in the ^{3}P state under our experimental illumination conditions when Q_A is reduced or removed. Still, the rise and decay of fluorescence from ^{1}P can be observed (with approximately 50% less signal than in WT) in M71GL carotenoid-free RCs. Similarly, a weak 905 nm probe beam collinear with the excitation beam, used to detect the bleach of P under experimental conditions, detected ~50% bleach at 905 nm in M71GL RCs [33]. Thus, in M71GL RCs, approximately half of the RCs are in the ^{3}P state at 85 K with steady-state excitation, and this is the system we exploit to study ^{1}B dynamics in ^{3}P-containing RCs. These issues are absent for the heterodimer as the triplet yield has been reported to be very small [34], and we have confirmed this finding. For this reason it is not possible to study ^{1}B decay in ^{3}D-containing RCs.

It is our experience that the Q_A site is often not 100% occupied. To ensure that the Q_A site is occupied in cases where this is important, excess menadione was added. Menadione has been shown to bind strongly and exclusively to the Q_A binding site [23,35]. Wild-type RCs with unmodified Q_A showed ~80% bleach of the 905 nm probe beam. Addition of excess menadione increased this bleach to >95%; however, the kinetics of ^{1}B decay were the same with or without addition of excess menadione. Lifetimes and amplitudes in the text refer to samples without excess menadione added; however, the tables include the values for samples with menadione added for completeness.

3.2. Time-resolved fluorescence from ^{1}B in wild-type RCs containing P^+

For excitation of H at 760 nm, fluorescence from ^{1}B was monitored at 815 nm in four different samples of wild-type RCs at 85 K: (i) RCs containing P^+ formed by chemical oxidation with potassium ferricyanide; (ii) RCs containing unmodified Q_A; (iii) RCs containing Q_A that was reduced with sodium dithionite (Q_A-reduced); and (iv) RCs from which Q_A was removed (Q_A-depleted). Fig. 4 compares the ^{1}B decay of WT RCs, WT Q_A-reduced RCs, and P^+ WT RCs. The decay of ^{1}B in WT Q_A-depleted RCs is compared against the decay of ^{1}B in M71GL Q_A-depleted RCs in Fig. 5. For WT RCs containing chemically oxidized P, the ^{1}B decay can be fit with one dominant component with time constant (and amplitude) 271 ± 3 fs ($99.7 \pm 0.1\%$). The decay time constants for unmodified-Q_A and Q_A-reduced WT RCs are 261 ± 4 fs ($99.8 \pm 0.1\%$) and 189 ± 6 fs ($99.4 \pm 0.2\%$), respectively. For Q_A-depleted WT RCs, the decay of ^{1}B is slightly different; two decay components with lifetimes (and amplitudes) of 225 ± 8 fs ($95.2 \pm 0.5\%$) and >5 ps ($4.8 \pm 0.5\%$) are necessary to obtain a good fit to the data. The >5 ps component is poorly determined because we measure only the first 7.5 ps of the decay. The RC samples, their respective states, and their ^{1}B emission decay components are summarized in Table 1.

3.3. Time-resolved fluorescence from ^{1}B in M71GL RCs containing ^{3}P

For excitation of H at 760 nm, fluorescence from ^{1}B was monitored at 815 nm in Q_A-depleted M71GL RCs at 85 K (Fig. 5). One dominant decay component was observed with time constant

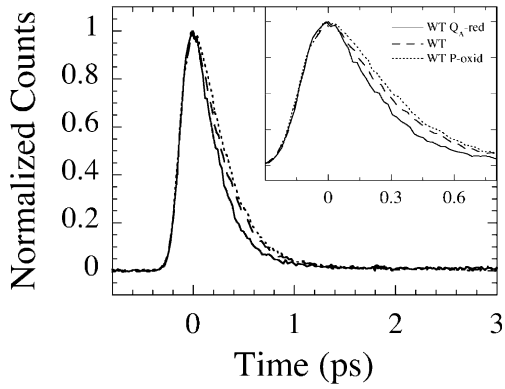

Fig. 4. Spontaneous fluorescence from wild-type Q_A-reduced RCs (solid), RCs with neither Q reduced or P oxidized (dashed) and P-oxidized RCs (dotted) measured at 815 nm following excitation in the H band at 760 nm at 85 K.

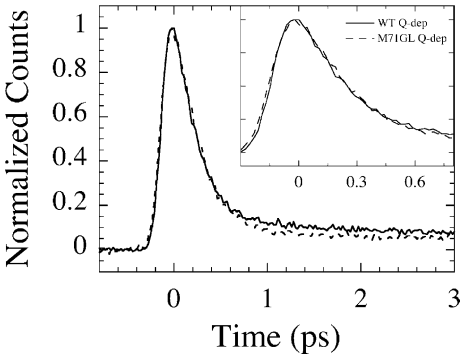

Fig. 5. Spontaneous fluorescence from wild-type Q_A-depleted RCs (solid) and M71GL Q_A-depleted RCs (dashed) measured at 815 nm following excitation in the H band at 760 nm at 85 K.

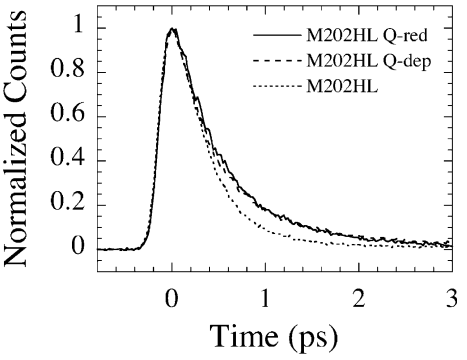

Fig. 6. Spontaneous fluorescence from M202HL Q_A-reduced RCs (solid), M202HL Q_A-depleted RCs (dashed) and M202HL RCs (dotted) measured at 815 nm following excitation in the H band at 760 nm at 85 K.

(and amplitude) 236 ± 19 fs ($96.8 \pm 0.5\%$), along with a minor long-lived component >5 ps ($3.2 \pm 0.5\%$) that is poorly determined because of the time window of the experiment. The M71GL ^{1}B emission decay components are reported in Table 1.

3.4. Time-resolved fluorescence from ^1B in M202HL heterodimer RCs

For excitation of H at 760 nm, fluorescence from ^{1}B was monitored at 815 nm in three different samples of heterodimer RCs at 85 K: (i) RCs in which Q_A was present; (ii) RCs in which Q_A was chemically reduced with sodium dithionite (Q_A-reduced); (iii) RCs in which Q_A was removed (Q_A-depleted) (Fig. 6). For Q_A-reduced M202HL RCs, two dominant decay components with time constants (and amplitudes) of 254 ± 24 fs ($66.7 \pm 2.7\%$) and of 782 ± 24 fs ($33.0 \pm 2.6\%$) are necessary to obtain a good fit to the data. Similar to Q_A-reduced RCs, for Q_A-depleted M202HL RCs there are two decay components with lifetimes (and amplitudes) of 238 ± 20 fs ($74.6 \pm 1.5\%$) and of 772 ± 28 fs ($24.2 \pm 1.5\%$). For M202HL RCs in which Q_A was not pre-reduced, i.e., D^+-containing RCs, the decay of ^{1}B is significantly different from the two cases above. For these RCs, essentially only one decay component with time constant (and amplitude) 343 ± 16 fs ($98.9 \pm 0.3\%$) is necessary to describe the data. All of the ^{1}B emission decay components for the M202HL mutant are reported in Table 2.

4. Discussion

4.1. Singlet energy transfer in P^+ containing wild-type RCs

Fleming and co-workers [4], Shuvalov and co-workers [36], and Woodbury and co-workers [37] measured a return of the bleach of the B band in R26 RCs containing a chemically-oxidized special pair that was not significantly longer than for RCs with P in the neutral ground state. Here we report similar findings looking at ^{1}B decay directly: the lifetime increases from 225 fs in Q_A-depleted wild-type RCs (P) to ~270 fs in RCs in which the special pair is chemically oxidized (P^+Q_A). Similarly, the lifetime increases from ~190 fs in Q_A-reduced wild-type RCs ($P\ Q_A^-$) to ~260 fs in wild-type RCs ($P^+Q_A^-$). Q_A-reduction itself seems to have the small but noticeable effect of increasing the $^1B \rightarrow P$ (or P^+) energy transfer rate. In wild-type RCs in which the special pair is missing, Woodbury and co-workers [37] and our lab [unpublished results] both observed a long lived ^{1}B state ($\tau \sim 800$ ps). Therefore, the presence of either neutral P or P^+ produces rapid quenching of the singlet excited state of B. The simplest conclusion is that ^{1}B transfers energy to P^+, and does so at

nearly the same rate as to P. Because we cannot probe the resulting excited state of P^+, we can only assume that it is formed and, as is the case with nearly all excited states of the radical ions of aromatic systems in the condensed phase, it relaxes to the ground state of P^+ very rapidly. No evidence is seen in the transient absorption experiments for a long-lived state associated with B caused, for example, by enhanced intersystem crossing to ^3B due to the proximity of the spin on P^+ [4,36,37]. As seen in Fig. 2C, the P^+ absorption band has virtually zero intensity in the Q_Y region where P absorbs, thus spectral overlap with the emission of ^1B, whose spectrum we have reported [8], is drastically reduced compared with spectral overlap with P. Hence, the rate of energy transfer between ^1B and P^+ seems not to depend appreciably on the oscillator strength of the acceptor.

4.2. Singlet energy transfer in ^3P-containing M71GL RCs

Fig. 5 shows that ^1B is quenched rapidly in Q_A-depleted M71GL mutant RCs. In the absence of triplet energy transfer from ^3P to the carotenoid, approximately half of excited M71GL RCs become trapped in the relatively long-lived ^3P state at steady state. There is no evidence of multiple ^1B decay components, which might occur if some RCs contained ground state P, others contained ^3P, and the rate of energy transfer to these two states were different. The ~3% long-lived component present in M71GL Q_A-depleted RCs is also present in WT Q_A-depleted RCs indicating that the long-lived fluorescence is not associated with energy transfer to ^3P but rather a result of Q_A-depletion itself (Fig. 5). The 236 fs lifetime of ^1B decay is essentially the same as that seen for WT Q_A-depleted RCs where P is in the neutral ground state. Therefore, as with P and P^+, the presence of ^3P produces rapid quenching of the singlet excited state of B. The simplest conclusion is that ^1B transfers energy almost as effectively to ^3P as to P and as effectively to ^3P as to P^+. Hence, the rate of energy transfer between ^1B and P, P^+ or ^3P seems not to depend (or to depend only weakly, vide infra) on the oscillator strength of the acceptor in the region of ^1B emission, indicating that the density of acceptor states is relatively conserved over this region.

4.3. Singlet energy transfer in D^+-containing M202HL mutant RCs

In previous work, we reported different energy transfer rates for $^1B_L \rightarrow D$ and $^1B_M \rightarrow D$ based on the observations that the ^1D emission rises and the ^1B emission decays with two well-resolved components [17,18]. Tuning the excitation wavelength across the H and B bands changes the relative contributions of these components in a manner consistent with faster energy transfer along the M branch and slower energy transfer along the L branch. In the work presented here, we monitor the ^1B decay kinetics to compare energy transfer to D in Q_A-reduced and Q_A-depleted heterodimer RCs with energy transfer to D^+ in RCs containing $D^+Q_A^-$ formed from the initial charge separation reaction at steady state by our excitation beam in Q_A-containing RCs. The decay of $^1B_{L,M}$ becomes mono-exponential in D^+ heterodimer RCs with a ~340 fs lifetime, slightly longer than the ~250 fs lifetime of $^1B_M \rightarrow D$. Hence, $^1B_L \rightarrow D^+$ becomes substantially faster than $^1B_L \rightarrow D$ while energy transfer from ^1B_M remains about the same.

4.4. Singlet energy transfer mechanism(s) in the RC

The data presented in this manuscript both confirm and extend experiments in our and other laboratories on the quenching of ^1B by modified energy acceptors. In the case of ^3P, P^+, and D^+, the absorption spectrum of the putative energy acceptor is drastically changed compared with the neutral ground state. Nonetheless, ^1B is quenched extremely rapidly. In the conventional Förster dipole–dipole mechanism, the spectral overlap between donor emission and acceptor absorption is a major factor that determines the rate of energy transfer. At the other extreme, the Dexter exchange mechanism describes the possibility of excited-state energy transfer even when the dipole strengths of both donor and acceptor are small (for example triplet energy transfer) so long as a sufficient density of acceptor states is present. In previous papers in this series, we also found that

the singlet energy transfer rate from the monomeric acceptors is surprisingly insensitive to either the spectrum of the donor (e.g., when a bacteriochlorophyll replaces bacteriopheophytin in the H_L binding site in the M214LH mutant [7], or a bacteriopheophytin replaces a bacteriochlorophyll in the B_M binding site in the M182HL mutant [8]) or the acceptor (e.g., when the temperature is lowered, leading to a shift of the absorption spectrum of P [7]). P^+ and ^3P are extreme cases because the absorption in the Q_Y region is drastically altered so that a conventional Förster analysis would predict a drastic reduction in ^1B quenching, contrary to what is observed. Since both P^+ and ^3P are open shell molecules, it is possible that the spin causes enhanced intersystem crossing converting ^1B to ^3B. However, transient absorption spectra of P^+ containing RCs (we are not aware of similar measurements on ^3P-containing RCs) show that the ground state of B is rapidly reformed [4,36,37]. It is conceivable that $^1B \rightarrow {}^3B \rightarrow B$ occurs on the hundreds of fs timescale in the presence of P^+ or ^3P, though this is much faster than what is expected.

Recently, calculations of energy transfer rates using a generalized version of Förster theory have been able to account for the ultrafast rates observed in wild-type RCs, as well as in the heterodimer and beta mutants [38,39]. Agreement between calculation and experiment relied on the density of states of the upper exciton band of P, unweighted by its oscillator strength, as the primary acceptor of singlet energy, as well as improved electronic coupling calculation methods and the use of experimentally determined line shapes. In the case of the heterodimer mutant, agreement between calculation and experiment depended upon a reduction in the electronic couplings between D_L and the other RC pigments. Even within such a framework, however, it is not clear how the extremely rapid energy transfer rates from B to oxidized and triplet special pairs can be rationalized. Although the density of states of the acceptor state is unweighted according to the dipole strength of the transition, thereby allowing weakly allowed transitions to participate in efficient excited-state energy transfer, the overall coupling weighted spectral overlap integral is still highly dependent on the proximity of donor/acceptor state energies. However, it seems unlikely that this overlap integral for the oxidized and triplet acceptor states would remain unchanged compared to that calculated for the upper exciton band of P.

Considering conventional Förster analysis, it is not obvious what factors would be responsible for the asymmetry in energy transfer along the L and M sides in the M202HL mutant or the symmetry in those RCs containing $D^+Q_A^-$. Crystal structures of the wild-type and M202HL RCs do not show significant differences in the distances between or the orientations of the special pair dimer and accessory bacteriochlorophylls [40], although it is possible that the magnitude of structural changes required to influence energy transfer rates on the femtosecond timescale lies below the resolution of the current structures (~2.5–3.0 Å). ENDOR experiments on the M202HL mutant have shown that the unpaired electron hole on D^+ is localized on the bacteriochlorphyll, D_L [41], making the restoration of symmetry in energy transfer rates in D^+-containing RCs especially intriguing. In a general model of energy transfer that treats electronic energy transfer using a Coulombic interaction in the framework of the Fermi golden rule, what is required is electronic coupling and a sufficient density of states to conserve energy. The results presented here (as well as those of others on P^+ RCs) suggest that the density of states is always sufficient. The electronic interactions between the chromophores must be important and the results from the heterodimer mutant suggest that their contribution can dominate the energy transfer rate. We have perturbed the electronic structure of the special pair and simultaneously diminished the spectral overlap between ^1B and the "perturbed special pair" absorption in wild-type, M71GL, and the heterodimer mutant RCs. Nevertheless, $^1B \rightarrow P^+$ and $^1B \rightarrow {}^3P$ energy transfers occur with nearly the same rate as $^1B \rightarrow P$ in wild-type and M71GL RCs, respectively. In M202HL RCs containing D^+, however, the effect is dramatic with the lifetime of $^1B_L \rightarrow D^+$ energy transfer being much shorter than $^1B_L \rightarrow D$. It is significant that in *every* case the donor/acceptor spectral overlap appears to be altered. A conventional Förster analysis

predicts that energy transfer should slow down or remain the same if there is sufficient density of states (even with the apparent loss of spectral overlap). However, we observe that energy transfer *speeds up* in the heterodimer mutant along the L branch. One explanation is that the perturbation to the electronic interactions conspires to enhance the rate of $^1B_L \rightarrow D^+$ energy transfer over $^1B_L \rightarrow D$ energy transfer. Such an effect suggests that only a mechanism that takes into account electronic interactions between chromophores will adequately describe energy transfer in the reaction center.

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