Antenna Excited State Decay Kinetics Establish Primary Electron Transfer in Reaction Centers as Heterogeneous†

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ABSTRACT: The decay of the excited primary electron donor P* in bacterial photosynthetic reaction centers (both membrane-bound and detergent-isolated) has been observed to be nonexponential on a time scale of some tens of picoseconds. Although the multipicosecond nonexponentiality of P* has been ascribed to heterogeneity in the rate of primary electron transfer (PET), the decay kinetics can be interpreted equally well using homogeneous models. To address this ambiguity, we studied the decay of excited bacteriochlorophyll (Bchl) in the membrane-bound core antenna/reaction center complexes of wild-type and mutant reaction center strains of *Rhodobacter capsulatus*. Reaction centers isolated from these same strains display a range of multiexponentiality in primary charge separation. The mutant strains carry substitutions of amino acids residing near the monomeric Bchl on the active and/or inactive sides of the reaction center. Transient absorption measurements monitoring the Q_y bleach of antenna Bchls require at least two exponential components to fit all decays. The wild type was fitted with equal-amplitude components whose lifetimes are 24 and 65 ps. The shortest-lived component is relatively insensitive to mutation, in contrast to the longer-lived component(s) whose amplitude and magnitude were dramatically perturbed by amino acid substitutions. Unlike the situation with isolated reaction centers, here the only kinetic models consistent with the data are those in which the primary electron-transfer rate constant is heterogeneous, suggesting at least two structural populations of RCs. PET in the population with the shortest-lived antenna decay causes the kinetics to be transfer-to-trap-limited, whereas the kinetics in the other population(s)—having longer-lived antenna decays—are limited by the rate of PET. Observation of both types of kinetic limitation within a single light-harvesting system is unexpected and complicates any discussion of the rate-limiting step of light energy utilization in photosynthesis.

In purple, non-sulfur photosynthetic bacteria, photoautotrophic growth is facilitated through the absorption of photons by specialized light-harvesting complexes known as antennae. These transmembrane pigment-protein complexes, which are localized to unique invaginations (chromatophores) of the inner cell membrane, bind the majority of bacteriochlorophyll (Bchl)[1] in the cells. Many photosynthetic bacteria synthesize two unique antenna complexes which are differentiated based upon their proposed relative location with respect to the reaction center (RC) where primary charge separation occurs. A structural model for the inner (core) antenna, known as LH1, suggests that it contains one Bchl array consisting of 30−40 molecules (Karrasch et al., 1995; Papiz et al., 1996). Recent structures for the outer (peripheral) antenna, known as LH2, shows that it accommodates two arrays of either 9 and 18 (McDermott et al., 1995; Savage et al., 1996) or 8 and 16 Bchl molecules (Koepke et al., 1996). The configurations of the Bchl arrays, as well as their interaction with the protein matrices, tune the energy levels of the different Bchls in such a way that electronic excitation flows energetically downhill from the outer LH2 to the inner LH1 (Cogdell et al., 1996). The RC, whose structure is also known to atomic resolution (reviewed in Ermler et al., 1994), consists of three transmembrane subunits—L, M, and H—and several prosthetic groups.

In the RC, primary charge separation is initiated on a specialized dimer of Bchl known as the special pair (P). Primary charge separation follows the arrival of an excitation from the LH1 antenna at P, forming the state P*, and

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[1] Abbreviations: RC, reaction center; Bchl, bacteriochlorophyll; P, special pair Bchls; LH1, light-harvesting complex 1; LH2, light-harvesting complex 2; *Rb.*, *Rhodobacter*; ps, picosecond; fs, femtosecond; OPA, optical parametric amplifier; PET, primary electron transfer; k_i, photochemical rate constant; k_T, trapping rate constant; k_D, detrapping rate constant; k_A, excited state energy transfer rate constant in the antenna; q_{eff}, effective coordination number for trapping; N, number of Bchl molecules per RC in LH1; OAS, one antenna site; PMS, phenazine methosulfate.

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proceeds with electron migration from P* to a bacteriopheophytin molecule (H_A), forming the state $P^+H_A^-$ with an average time constant of ≈3 ps. In subsequent steps, the electron is transported to the primary quinone (Q_A; in 200 ps) and finally to the terminal acceptor, the secondary quinone (Q_B; in 5−200 μs). The charge separation reactions build up a proton gradient across the membrane which is used to drive ATP synthesis.

In studies employing both time-resolved absorption and fluorescence techniques, the decay of the excited primary electron donor P* in RCs isolated from several species of photosynthetic bacteria has been observed to be nonexponential on a time scale of some tens of picoseconds. The nonexponentiality of the P* decay has been observed in both wild-type (WT) and mutant RCs from *Rhodobacter (Rb.) capsulatus* (Chan et al., 1991; Du et al., 1992; Jia et al., 1993) and *Rb. sphaeroides* (Kirmaier & Holten, 1990; 1991; Müller et al., 1992; Nagarajan et al., 1993; Woodbury et al., 1994; Peloquin et al., 1994; 1995; 1996; Holzwarth & Müller, 1996; Lin et al., 1996). Although observed in *Rhodobacter*, nonexponentiality of the P* decay on a time scale of some tens of picoseconds has never been resolved for RCs of *Rhodopseudomonas (Rps.) viridis* or *Rhodospirillum (Rsp.) rubrum*. Decays of P* in *Rps. viridis* were reported to be monoexponential (Wasielewski & Tiede, 1986; Breton et al., 1986); the P* decay in *Rsp. rubrum* RCs was shown to be monoexponential in a study with Q_A-reduced RCs that also yielded monoexponential P* decays for RCs of *Rb. sphaeroides* and *Rb. capsulatus* (Wang et al., 1994).

Nonexponentiality of the P* decay is not unique to detergent-isolated RCs, because it has also been reported for membrane-bound RCs of LH1- and LH2-deficient strains of *Rb. sphaeroides* (Beekman et al., 1995) and more recently *Rb. capsulatus* (Müller et al., 1996). These data suggest that detergent isolation of the RC complex is not inducing the heterogeneity. Any discussion of the temperature dependence of the initial electron transfer reactions in bacterial RCs can be impacted by these observations of heterogeneity, since observed temperature dependencies may be due to changes in distinct structural populations rather than electron transfer properties (Kirmaier & Holten, 1990; 1991; Nagarajan et al., 1990; 1993; Peloquin et al., 1996).

The cause of the nonexponentiality has been difficult to determine unambiguously. Some groups have attributed it to homogeneous origins involving internal P* or radical pair relaxation (Nagarajan et al., 1993; Lin et al., 1996) or solvent/protein relaxation (Peloquin et al., 1994; 1995; 1996) on the time-scale of primary charge separation, or branching of the initial electron transfer ($P^* \rightarrow P^+H^-$) to involve one (or both) Bchl monomers as intermediates (Müller et al., 1992). Other studies have found that the temperature dependence of the nonexponentiality could fit well with a heterogeneous model involving discrete structural populations of RCs with different inherent rates of primary charge separation (Kirmaier & Holten, 1990; 1991; Müller et al., 1992).

Using a simple theoretical model, Pearlstein (1996a) recently showed that the combined LH1−RC system can, in principle, be used to discriminate between homogeneous and heterogeneous causes of the nonexponential multipicosecond decay of P* in RCs, even though kinetic discrimination (with adjustable parameters) is impossible using signals from isolated RCs themselves (Müller et al., 1992). It is the extra "kinetic dimension" afforded by the exciton transfer between antenna and RC that enables this method. Except under conditions that would be extreme for a photosynthetic system, the zero-mode-dominance principle (Pearlstein, 1982) applies to each homogeneous subpopulation of RCs *separately*. Thus, if primary electron transfer (PET) is kinetically homogeneous over the entire population of RCs, even if it is multistep in each RC, the decay of the LH1 (Bchl) excited state should be essentially monoexponential as long as the kinetics of exciton trapping by the RC are not rate-limited by the process of exciton diffusion within LH1 itself. The same conclusion holds for each homogeneous subpopulation within an overall heterogeneous population of RCs. This means, for example, that if the overall population is bimodally heterogeneous (i.e., having exactly two homogeneous subpopulations of PET kinetics), one expects to observe a biexponential decay of the energetically coupled antenna excitation (LH1*). More generally, one expects the LH1* decay to depend on the functional distribution of the PET kinetics over the entire population of RCs.

The decays of excited states in coupled LH1/RC complexes have been reported for a variety of species of photosynthetic purple non-sulfur bacteria whose phenotypes differ with respect to LH2 (Table 1). Some of the species (*Rsp. rubrum* and *Rps. viridis*) naturally lack LH2, whereas others have been altered genetically (*Rb. capsulatus* and *Rb. sphaeroides*) or grown under conditions in which the LH2 genes are not expressed (*Rps. cryptolactis*; high light). The majority of the studies utilize the methods of one- or two-color transient absorption (pump-probe) or single photon counting with 3 to 20-ps or 50 to 70-ps time resolution, respectively. An exception is the study by Xiao et al. (1994) on *Rb. capsulatus*, which uses a two-color transient absorption spectrophotometer with 150-fs resolution. The decays of excited states in LH1/RC complexes in wild-type strains are in remarkable agreement across species. With RCs in the "open" state (PHQ), decays are reported to be monoexponential with lifetimes between 35 and 60 ps (Table 1). Most measurements on wild-type samples report small fractions of longer-lived components (between 140 and 250 ps) which are assigned to quenching by RCs in the "closed" state (PHQ^-; see Table 1 for references).

Studies exploring excited state decays in RC mutant strains report little if any correlation between the rates of LH1/RC decay and primary charge separation (measured on isolated RCs from the same strains; Beekman et al., 1994; Freiberg et al., 1996). The conclusions drawn from the lack of correlation are that the overall trapping kinetics in bacterial photosynthetic units are limited by rates of energy transfer rather than electron transfer.

In this study we explore the utility of the theory recently described by Pearlstein (1996a) by examining LH1/RC decays in a well-characterized family of RC mutants of *Rb. capsulatus*. The wild-type and mutant strains lack LH2 and thus synthesize only the LH1 complex binding 30−40 bacteriochlorophyll molecules. These mutant strains carry substitutions of amino acids in the RC at L181 and M208 (M210 in *Rb. sphaeroides*). These residues are located near the monomeric Bchls on the inactive and/or active branches of the RC cofactors, respectively. Primary electron transfer rates have been determined for isolated RCs from this mutant family using either stimulated (Chan et al., 1991; Jia et al., 1993) or spontaneous (Du et al., 1992) emission to monitor

Table 1: Summary of Studies Exploring Excited State Decays in the Core Light-Harvesting Antenna (LH1) from Membrane Preparations of Photosynthetic Purple Bacteria. The Membranes either Naturally, Genetically, or Environmentally Lack the Peripheral LH2 Light-Harvesting Complex. Excited States in the Core Antenna Are Quenched by Wild-Type or Mutant Reaction Centers

reference	species	origin of $LH2^-$ phenotype	reaction center sequence	experimental technique	pump λ (nm)	probe λ (nm)	time resolution (ps)	decays measured (ps)	assignment of decay components
Timpmann et al., 1993	*Rsp. rubrum*	natural	wild type	streak camera and pump-probe	590, 795−870	900−910	15−20	60	quenching by open RCs
Freiberg et al., 1995; 1996	*Rsp. rubrum*	natural	wild type	single photon counting	590, 860	890	50−70	60	quenching by open RCs
Xiao et al., 1994	*Rb. capsulatus*	genetic	wild type	pump-probe (two color)	800, 880	750−905	0.15	36; globally analyzed	quenching by open RCs
Beekman et al., 1994	*Rb. sphaeriodes*	genetic	wild type and mutations at M210[a]	pump-probe (one color)	861	861	2−3	46, 250 (wt)	quenching by open RCs and feedback from secondary ET
Freiberg et al., 1995; 1996	*Rb. sphaeroides*	genetic	wild type and mutations near special pair	single photon counting	590, 860	890	50−70	60 (wt)	quenching by open RCs
Kramer et al., 1995	*Rsp. cryptolactis*	growth; high-light	wild type	pump-probe (one color)	900	900	4−6	50, 190	quenching by open and closed RCs
Timpmann et al., 1995	*Rps. viridis*	natural	wild type	single photon counting	810−890	1040	60−70	60, 140	quenching by open and closed RCs
present study	*Rb. capsulatus*	genetic	wild type and mutations at L181, M208	pump-probe (two color)	595	890	0.15	20, 60 (wt)	quenching by open RCs (heterogeneous PET)

[a] M208 in *Rb. capsulatus*.

the loss of P* following excitation by short (≤1 ps) laser pulses. The wild-type and mutant strains display a wide range of nonexponential electron transfer kinetics and are listed in Table 2 in order of increasing average P* lifetime, τ_{P^*ave}, where $\tau_{P^*ave} = \Sigma A_i \tau_i$. Primary electron transfer has been studied in similar mutants (Table 3) in both *Rb. sphaeroides* (Finkele et al., 1990; Nagarajan et al., 1990; 1993) and *Rps. viridis* (Bibikova et al., 1995). The P* decays from identical mutants in different species are remarkably similar, strongly supporting the data from *Rb. capsulatus*. In addition, the structure of one of the mutants of this family in *Rb. sphaeroides*, M210Tyr → Phe, is known to 3.0 Å resolution (Chirino et al., 1994), and the effects of local electrostatics in the region of the cofactors involved in primary charge separation have been treated by theoretical calculation (*Rps. viridis*; Parson et al., 1990).

In this paper, we show that the decays of the LH1 excited state from all strains studied (including the wild type) are nonexponential. The shortest-lived component is relatively insensitive to mutation, whereas the longer-lived components are sensitive to mutation and closely track increases in amplitudes and lifetimes observed in long-lived P* decay components seen in isolated RCs. The data, in conjunction with theoretical modeling, show conclusively that the nonexponentiality observed in primary electron transfer can only be explained by a heterogeneous distribution of PET rate constants indicating different structural populations.

EXPERIMENTAL PROCEDURES

Construction and Growth of Mutant Strains. The system for mutagenesis of *Rb. capsulatus* RC genes has been described (Youvan et al., 1985; Bylina et al., 1986; 1989). Oligonucleotide-directed site-specific mutagenesis by the method of Kunkel et al. (1987) was performed according to directions from a kit (BioRad) on L or M gene fragments (*Hin*dIII → *Kpn*I, *Kpn*I → *Bam*HI, respectively; Bylina et al., 1986) that had been subcloned into the bifunctional vector $pBS^+/-$ (Stratagene). The first mutants made at each site created new restriction sites, *Bal*I at codons L181−182 and *Sna*BI at M208−209. Single-stranded versions of tagged plasmids were used as templates for codon replacements at M208 and L181, and mutants were detected by screening for loss of the appropriate restriction site. Following confirmation of the mutation by dideoxy sequencing of double-stranded DNA, the mutant L or M gene was returned to the *puf* operon in plasmid pU29 (Bylina et al., 1986), and the mutant operon was switched for the wild-type fragment in broad-host-range plasmid pU2922 (Bylina et al., 1989). Mutant derivatives of pU2922 were transferred to *Rb. capsulatus* deletion strain U43 (Youvan et al., 1985; $LH1^-$, $LH2^-$, RC^-) via conjugation with *E. coli* donor strain S17−1 (Simon et al., 1983). The "wild-type" strain [$LH1^+$, $LH2^-$, RC^+] is U43 complemented *in trans* by plasmid pU2922. The M250Trp → Phe mutant strain (Coleman et al., 1990) was a generous gift from D. C. Youvan.

All strains were grown under chemoheterotrophic conditions (semiaerobic, dark, 34 °C) on RPYE medium (Hanson et al., 1992) containing kanamycin. Chromatophores were prepared from solution cultures according to the method of Prince & Youvan (1987) with modifications described below. Briefly, cells were washed and sonicated in 10 mM Tris (pH 7.3) and subsequently disrupted with a French press.

Table 2: Measured Rates of Primary Electron Transfer in Mutant Bacterial Photosynthetic Reaction Centers[a] and Results of Fits to Excited State Decays in Mutant Chromatophores. Strains Are Listed in Order of Increasing Average P* Lifetime, τ_{P^*ave}, where $\tau_{P^*ave} = \Sigma A_i\tau_i$[b]

		P* decay		LH1* decay							
strain (reps)	mutations	τ, ps; (A,%)	average P* lifetime, ps	τ_1, ps	A_1, %	τ_2, ps	A_2, %	τ_3, ps	A_3, %	τ_{ave}, ps	$1/(dP_{obs}/dt)$ at $t = 0$,[c] ps
M250F (2)	M250Trp → Phe			17.0 ± 1.4	29.3 ± 6.4	52.5 ± 0.7	70.7 ± 6.4	–	–	42	32.6 ± 3.0
WT (3)	**wild type (L181Phe, M208Tyr)**	**2.7 (72) 11.1 (28)**	**5.0**	**23.7 ± 2.1**	**51.0 ± 5.3**	**64.7 ± 4.6**	**49.0 ± 5.3**	–	–	**44**	**34.4 ± 1.5**
YY (2)	**L181Phe → Tyr**	**2.3 (84) 10.5 (16)**	**3.6**	**25.0 ± 2.8**	**49.4 ± 2.7**	**65.6 ± 2.1**	**50.7 ± 2.6**	–	–	**46**	**36.4 ± 4.5**
HH (2)	**L181Phe → His M208Tyr → His**	**4.4 (100)**	**4.4**	**30.5 ± 3.5**	**60.3 ± 4.0**	**99.5 ± 12.0**	**39.8 ± 4.0**	–	–	**58**	**42.1 ± 4.6**
YF (3)	**L181Phe → Tyr M208Tyr → Phe**	**3.5 (60) 24.2 (40)**	**11.9**	**26.0 ± 5.2**	**47.7 ± 2.7**	**92.7 ± 6.5**	**52.3 ± 2.7**	–	–	**61**	**41.7 ± 4.5**
FF (3)	**M208Tyr → Phe**	**5.4 (53) 40.0 (47)**	**21.6**	**18.7 ± 2.1**	**30.2 ± 5.1**	**98.7 ± 3.2**	**69.8 ± 5.1**	–	–	**74**	**43.1 ± 1.0**
FE (2)	M208Tyr → Glu			17.0 ± 4.2	30.8 ± 2.1	106.0 ± 8.5	69.1 ± 2.2	–	–	78	40.6 ± 7.2
FK (2)	M208Tyr → Lys			26.0 ± 1.4	37.0 ± 0.8	120.5 ± 4.9	63.1 ± 0.8	–	–	85	51.4 ± 2.6
FT (2)	**M208Tyr → Thr**	**10.0 (38) 70.0 (62)**	**47.2**	**13.5 ± 3.5**	**24.5 ± 3.5**	**97.5 ± 27.5**	**50.1 ± 14.6**	**236 ± 91**	**25.5 ± 10.6**	**112**	**41.0 ± 14.4**

[a] Rates of primary charge separation in strains of *Rb. capsulatus* measured by the decay of P* using a femtosecond pump-probe system based on a CPM dye laser and 20 Hz dye amplifier (Jia et al., 1993). All measurements were collected at room temperature (290−296 K). [b] Rows with bold entries represent strains with measured rates of P* decay. All LH1* lifetimes reported are straight from nonlinear fitting routines ignoring the influence of the intrinsic (nonquenched) LH1 lifetime (0.75−1 ns) on the measured (quenched) excited state decays. We argue that these corrections are small (≈2.5% for τ_1 and ≤14% for τ_2) for lifetimes observed in wild-type and mutant strains. [c] The negative, inverse initial slopes of ΔA_{890} are represented as $1/(dP_{obs}/dt)_{t=0}$ (eq 1).

Table 3: Primary Electron Transfer in the L181/M208[a] Family of Mutants from Different Species of Purple Non-Sulfur Photosynthetic Bacteria

strain designation	mutation(s)	P* decay [τ, ps; (A,%)]		
		Rb. capsulatus	*Rb. sphaeroides*	*Rps. viridis*
YY	L181Phe → Tyr	2.3 (84)/10.5 (16)[b]	2.1 (94)/15 (6) [j]	1.7[f]
HH	L181Phe → His M208Tyr → His	4.4[b]		
WT (wild type)	(L181Phe, M208Tyr)	2.7 (72)/11.1 (28);[b] 4.5[h]	3.5;[b] 3.5;[c] 6.1[h]	3.5;[e] 2.8;[f] 5.9[e]
YF	L181Phe → Tyr M208Tyr → Phe	3.5 (60)/24.2 (40)[b]	3.5 (63)/18 (37)[j]	8.2[f]
FF	M208Tyr → Phe	5.4 (53)/ 40.0 (47)[b]	10.5;[c] 16 (75)/70 (25);[d] 6.1 (42)/26 (58)[j]	20[f]
FI	M208Tyr → Ile		16[c]	
FL	M208Tyr → Leu		22 (75)/90 (25)[d]	
FH	M208Tyr → His	3.90 (100)[b]		
TT	L181Phe → Thr M208Tyr → Thr	8.1 (52)/ 56.3 (48)[b]		
FT	M208Tyr → Thr	10.0 (38)/70.0 (62)[b]		
FW	M208Tyr → Trp		41[i]	

[a] M210 in *Rb. sphaeroides*. [b] Jia et al., 1993; P* decay; Q_A oxidized RCs; 290−296 K. [c] Nagarajan et al., 1990; P* decay; Q_A oxidized RCs; 295 K. [d] Gray et al., 1990; P* decay; some Q_A containing/some Q_A removed RCs; 298 K. [e] Wasielewski & Tiede, 1986; H_A signals; Q_A reduced RCs; 295 K. [f] Bibikova et al., 1995; P* decay; Q_A presence/redox state and temperature unkown. [g] Breton et al., 1986; P* decay; H_A signals; Q_A presence/redox state and temperature unkown. [h] Wang et al., 1994; P* decay; Q_A reduced RCs; 295 K. [i] Nagarajan et al., 1993; P* decay; Q_A oxidized RCs; 295 K. [j] Hamm et al., 1993; P* decay; Q_A oxidized RCs; 298 K.

Membrane fragments were recovered by ultracentrifugation at 4 °C of lysate devoid of cell debris. Chromatophore pellets were resuspended using a small paint brush and cold tissue homogenizer (Teflon piston/glass barrel unit) and diluted in cold Tris buffer (pH 7.3) to an OD at 875 nm of 2.0 in a 1-cm pathlength cell. Samples were used immediately or stored at −80 °C. Steady-state absorption spectra were recorded using a Shimadzu Scientific Instruments, Inc. (Columbia, MD) Model UV1601-PC spectrophotometer before and after laser excitation. No differences between fresh or frozen samples were observed in steady-state absorption spectra or excited state decay kinetics.

For the transient absorption measurements, samples were thawed and centrifuged at 6000 rpm for 1−3 min to remove large, light-scattering aggregates. Supernatant (1.5 mL) was added to 4-mL (1-cm pathlength) cuvettes along with phenazine methosulfate (PMS; in ethanol) and ascorbic acid to final concentrations of 50 μM and 25 mM, respectively. The final concentration of ethanol was <1%.

Details of the experimental setup for the femtosecond transient absorption measurements are given in Greenfield et al. (1996). Briefly, sub-200-fs excitation pulses at 595 nm (∼6-nm bandwidths) were provided by a 1.3-kHz optical parametric amplifier (Greenfield & Wasielewski, 1995). The

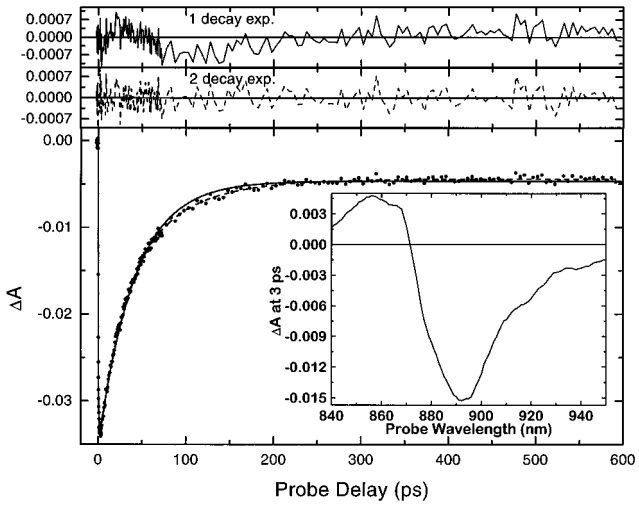

FIGURE 1: Example of a typical kinetic trace with nonlinear fits to excited state decays in LH1/RC complexes of the wild-type strain. The top panels show the residuals for one- and two-exponential decay fits with nonreduced χ^2 of 9.66×10^{-7} and 5.90×10^{-7}, respectively; inset depicts transient spectrum at ≈3 ps.

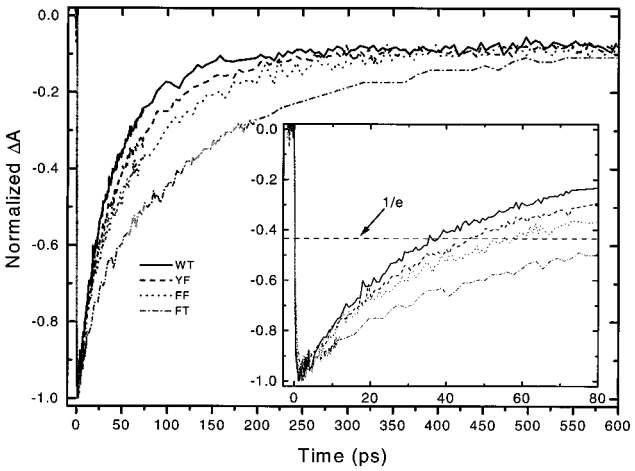

FIGURE 2: Representative decays (normalized to maximum signal at $t = 3$ ps) of excited states in LH1 antenna from wild-type and mutant reaction center strains. Strain designations are as in Table 2. The similarity of the initial slopes of the decays are revealed in an expanded time axis shown in inset. The average LH1 excited state decay may be estimated from the time at which the signal passes through $1/e$ (≈43%; inset) adjusted for the long-lived ΔA observed in these measurements.

white light continuum probe was generated by focusing the 835-nm fundamental into a sapphire plate. An 850-nm long-pass interference filter eliminated the "blue" side of the continuum and the residual light at 835 nm. The polarization of the probe was at the magic angle with respect to the pump. Pump and probe spot sizes were ~250 μm (diameter), and excitation energies were limited to 25 nJ to avoid multiple excitation of individual LH1/RC complexes. The sample was rapidly stirred to avoid reexcitation of the chromatophores by the next pump pulse. Using simple statistical simulations previously described (Greenfield et al., 1996) with 0.16 photons per LH1/RC complex, we estimate that greater than 93% of the complexes which absorb a photon are singly excited; less than 7% of those excited receive two or more photons.

Data were fitted to a sum of exponentials using the nonlinear curve-fitting routines built into the software package Origin (Microcal Software, Inc., Northhampton, MA). The quality of the fits was judged by the distribution of residuals and magnitude of the χ-square merit function.

RESULTS

Steady-state absorption spectra of wild-type and mutant chromatophores revealed no significant differences in the ratio of relative optical densities at 870 versus 800 nm, indicating that the assembly of LH1/RC complexes is not impaired by any of the RC mutations (data not shown). The transient spectrum (at 3 ps) of the wild-type chromatophore samples in the near-IR when excited with 595-nm light (Figure 1; inset) closely resembles that previously reported (Xiao et al., 1994). The spectrum has a derivative shape with positive region at wavelengths between 840 and 870 nm and negative region from 875 to 930 nm, with the zero-crossing point at 871 nm. The positive feature is dominated by excited state absorption of LH1-Bchl whereas the negative region is dominated by stimulated emission of LH1-Bchl (and to a lesser extent bleach of the ground state spectrum; Xiao et al., 1994). The transient spectra of all mutant strains studied mimic that of the wild type (data not shown).

In order to describe completely the multipicosecond transients recorded at 890 nm, fits to the data required one rising component and at least two decaying components. The rising component (averaging between 400 and 500 fs for the wild type) was not influenced by RC mutation. Although this rise-time is several times slower than the width of the excitation pulse, it may represent the instrument response of the system using 595-nm pump and 890-nm probe pulses with a 1-cm sample cell. Dispersion within the sample (i.e. different group velocities for the pump and probe pulses) causes temporal overlap between the pump and probe to occur at different delay times for different longitudinal positions within the sample. This results in a degradation of the system time resolution, and the estimated effect of the dispersion of 1 cm of water is consistent with the measured rise time. However, we cannot exclude a contribution to the rise time due to electronic relaxation from the initially excited Bchl S_2 state to the S_1 state, which would result in delayed stimulated emission at the probe wavelength. Direct excitation of the Q_y peak, which eliminates any effects from electronic relaxation and minimizes the effects of sample dispersion, results in an instrument-limited 150-fs rise-time (unpublished results). Subsequent discussion will be limited to the analysis of the decaying components.

Typical one- and two-exponential fits to wild-type excited state decay kinetics obtained with 890-nm probe are shown in Figure 1. The superior quality of the two-exponential decay fit is reflected in the distribution of residuals (Figure 1) and in a significantly reduced χ^2 value. The two-exponential decay fit returned lifetimes of 24 and 65 ps with equal amplitude (Table 2).

Figure 2 shows pictorially the wide range of LH1 excited state decays observed in the mutants studied. Table 2 outlines the results of multiexponential fits to the data as averaged over both laser and sample replications. Strains are listed in order of increasing average LH1* lifetime, τ_{ave} (where $\tau_{ave} = \Sigma A_i \tau_i$ with sum taken over all decay components). Results reveal that the longer-lived components (between 50 and 240 ps) are quite sensitive to RC mutation, whereas the shortest-lived component (between 13 and 30 ps) is relatively less sensitive. As one progresses through the mutants (in the order of increasing average lifetime of

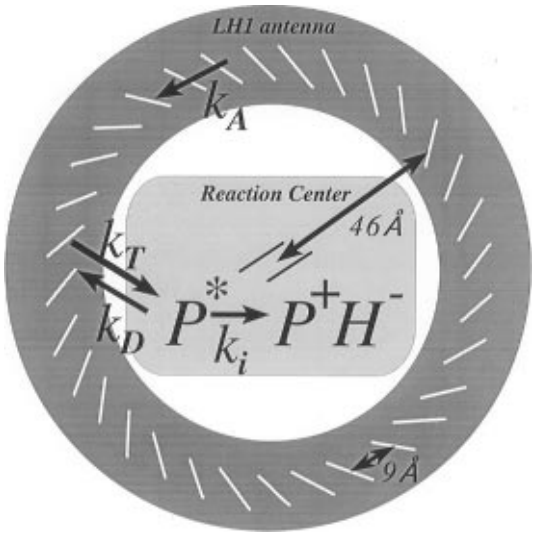

FIGURE 3: Kinetic description of excited state energy and electron transfer in LH1/RC complexes contained within chromatophores of purple, non-sulfur bacteria. Rate constants in the model are defined as follows: k_A, nearest-neighbor excited state energy transfer in LH1; k_T, excited state energy transfer from LH1 to the RC (trapping); k_D, excited state energy transfer from the RC to LH1 (detrapping); and k_i, primary electron transfer in the RC. Any one of the four rate constants can limit the overall trapping of excited state energy within LH1/RC complexes. These energy trapping bottlenecks are uniquely defined in the literature and are summarized here: PET limited ($k_i \ll k_T$, k_D, and k_A); diffusion limited ($k_A \approx k_T \approx k_D \ll k_i$); and transfer-to-trap limited ($k_T \approx k_D \ll k_A$ or k_i).

P* in isolated RCs; Table 2), the trend is an increasing average lifetime of LH1* (τ_{ave}; Table 2), attributed not only to increasing amplitudes but also to increasing lifetimes of the longer-lived components.

In order to judge the effect of mutation on parameters (here specifically, the rate of excited state energy migration from LH1 to the RC) important for modeling of the data, the negative, inverse initial slope was calculated from each fit as

$$-1/(\mathrm{d}P_{obs}/\mathrm{d}t)_{t=0} = \left[\sum_i \frac{A_i}{\tau_i}\right]^{-1} \quad (1)$$

where $P_{obs}(t)$ is the observed decay curve normalized so that $P_{obs}(0) = -1$. Negative, inverse initial slopes averaged over replications for the wild-type and mutant strains are shown in Table 2. Within the error of the measurement, the initial slopes of the LH1* decays are identical. The average of $-1/(\mathrm{d}t/\mathrm{d}P_{obs})_{t=0}$ across all strains studied is 40.4 ps.

DISCUSSION

Our understanding of the decays of excited states in LH1/RC complexes is now aided by structural characterization of both the LH2 of *Rps. acidophila* (McDermott et al., 1995), *Rsp. molischianum* (Koepke et al., 1996), and *Rhodovulum sulfidophilum* (Savage et al., 1996) and the LH1 of *Rsp. rubrum* (Karrasch et al., 1995). Structural models have been proposed for the arrangement of light-harvesting and reaction center superassemblies (Karrasch et al., 1995; Papiz et al., 1996; Pullerits & Sunström, 1996; Hu et al., 1997). These studies place the RC inside a ring (radius = 46 Å) of LH1-Bchl with P of the RC positioned at the midpoint of the structure. The center-to-center distance between neighboring Bchl monomers in the LH1 ring is ≈9 Å in these models. A summary of these similar LH1/RC models is presented as Figure 3.

Within the LH1/RC model structure (Papiz et al., 1996), assuming dipolar coupling (Förster, 1965) of pigments with a distance (R) dependence on the order of $1/R^6$, the single-step, excited-state hopping time between neighboring Bchls (or aggregates thereof) in the antenna is much shorter than that between the antenna and the reaction center Bchls. With intra-LH1 nearest neighbor Bchl separation of 9 Å, the intra-antenna Förster rate constants are 10^4-fold greater than the transfer-to-trap rate constant from the excited Bchl in LH1 to P* in the RC (if other factors in the Förster formula are the same). The energy transfer rate constants that arise from this arrangement of pigments clearly validates the use of a one-antenna-site (OAS) model in this work. If the intra-LH1 exciton motion is totally incoherent (Förster limit), OAS is completely equivalent to N antenna sites as long as the exciton trapping kinetics are far from the diffusion limit (Pearlstein, 1972). A similar conclusion holds on time scales of at least several picoseconds if the exciton motion is partially coherent (Hemenger et al., 1974; Pearlstein, 1996b). This streamlined description of the system simplifies the kinetics and allows for analytic solutions (with a limited number of variables) to the relatively complex set of differential equations. In its simplest form the OAS model of Pearlstein (1996a) may be expressed in terms of two coupled rate equations for each homogeneous subpopulation:

$$\frac{\mathrm{d}P_A(t)}{\mathrm{d}t} = -k_T P_A(t) + k_D P_{P*}(t) \quad (2)$$

$$\frac{\mathrm{d}P_{P*}(t)}{\mathrm{d}t} = k_T P_A(t) - (k_i + k_D) P_{P*}(t) \quad (3)$$

In eqs 2 and 3, $P_A(t)$ and $P_P{*}(t)$ are the probabilities at time t that the excitation energy resides in LH1 and in the state P*, respectively. The quantity k_i is the rate constant for PET (assumed to have a single path in each RC) in the i subpopulation. The energy-transfer rate constants are given by $k_T = q_{eff}F_T/N$ and $k_D = q_{eff}F_D$, where F_T and F_D are the Förster rate constants for trapping and detrapping, respectively, q_{eff} is the effective coordination number for trapping, and N is the number of bacteriochlorophyll molecules per LH1. For $k_D/k_T \gg 1$ and $k_T^{-1} \approx 20$ ps, expected to be the case for virtually all photosynthetic bacteria, it is shown (Pearlstein, 1996a) that the amplitude of the shorter-lived of the two exponential components in the simultaneous solutions of eqs 2 and 3 is a few percent. Thus, for all practical purposes, each homogeneous subpopulation gives rise to a single exponential decay component. In the i subpopulation, the expected monoexponential LH1* lifetime is given by

$$\tau_i = k_T^{-1}\left(1 + \frac{k_D}{k_i}\right) \quad (4)$$

If $k_D/k_i \ll 1$, the lifetime is said to be transfer-to-trap limited; if $k_D/k_i \gg 1$, it is PET-limited.

It is quite possible for one subpopulation to be transfer-to-trap limited while another is PET-limited. Indeed, if the RC population is characterized by a continuous distribution, there may be a corresponding distribution of the τ_i that ranges

smoothly from one kinetic limit to the other. In the transfer-to-trap limit, all τ_i have magnitudes close to that of k_T^{-1}. In the PET limit, the lifetimes become roughly proportional to the k_i^{-1} and are all substantially larger than k_T^{-1}. Thus, if k_T^{-1} is constant (or at least much more narrowly distributed that k_i^{-1}), the theoretical model predicts that for a continuous distribution of the k_i the decay of LH1* has an exponential component of relatively short lifetime (k_T^{-1} itself) and a longer-lasting, nonexponential tail. The latter, of course, entirely reflects the physical distribution of the k_i. We have experimental evidence which suggests that k_T^{-1} is narrowly distributed in these strains (see below).

It must be stressed that if the PET kinetics were homogeneous over the entire population of RCs, the theoretical model (Pearlstein, 1996a) would predict monoexponentiality of the antenna excitation decay. This would be the case even if there were multiple electron-transfer pathways in each RC, provided the PET rate constants are the same for all RCs. It is heterogeneity of PET over the population of RCs, not complexity of otherwise homogeneous PET pathways within each RC, that gives rise to nonexponentiality of the antenna excitation decay. Thus, the theoretical model makes a unique prediction for the antenna excitation decay shape: single exponential for a single homogeneous population (even if there are multiple electron transfer pathways within each RC), truly multiexponential for two or more kinetically distinct subpopulations. Clearly, the data presented here are inconsistent with the theoretical prediction for the case of homogeneous PET kinetics.

It must be noted that neither the values of τ_1, nor those of $-1/(dP_{obs}/dt)_{t=0}$, listed in Table 2 are strictly interpretable as k_T^{-1} according to the theoretical model, although the former are much closer (see below). However, the data given in both columns are consistent with invariance of k_T^{-1} over the set of nine strains (including wild type). For τ_1 the average over the set is 21.9 ± 5.6 ps, while for $-1/(dP_{obs}/dt)_{t=0}$ the average is 40.4 ± 5.5 ps. We show elsewhere (P. D. Laible, S. R. Greenfield, M. R. Wasielewski, D. K. Hanson, & R. M. Pearlstein; unpublished results) that, to a very good approximation, the theoretical model gives the result

$$k_T^{-1} = -1/(dP_{obs}/dt)_{t=0}\left[\frac{(1-2\rho^p)(1-[1+(k_D/k_T)]\rho^e)}{1-\rho^p-\rho^e}\right] \quad (5)$$

provided that the theoretical functions $P_A(t)$ and $P_P^*(t)$ are calculated for the initial conditions $P_A(0) = 1 - \rho^e$ and $P_P^*(0) = \rho^e$, respectively.

In eq 5, ρ^e is the fraction of excitation photons that are absorbed directly by the RC itself rather than by LH1. Also in eq 5, ρ^p reflects the relative signal strength (i.e. $\Delta A_{890\ nm}$) of a single P* as compared to a single LH1*

$$\rho^p = \frac{s^P}{s^P + s^{LH1}} \quad (6)$$

where s^P and s^{LH1} are the per protein signal strengths of the RC and LH1, respectively. For 595-nm excitation we estimate that $\rho^e = 0.1$ based upon the ratio of Bchl bound in the RC versus LH1 (this assumes identical oscillator strengths for all Bchl at 595 nm). We find experimentally that $\rho^p \cong 0.15$ for our samples (unpublished results). With $\rho^e = 0.1$ and $k_D = 2k_T$, eq 5 gives $k_T^{-1} = 0.65[-1/(dP_{obs}/dt)_{t=0}]$. This ratio, 0.65, agrees reasonably well with the value of 0.54 obtained from the averages of the experimental values above (agreement is exact for $k_D/k_T = 3.2$.)

The fact that the standard deviation from the mean on a percentage basis is twice as great for τ_1 as for the negative initial slope (Table 2) requires further explanation. Possibly, this is a result of forcing a bi- or tri-exponential fit on a more highly nonexponential decay. The fitting routine varies the value of τ_1 until the "best fit" is achieved without regard to any external constraint, such as the value of k_T. This interpretation is reinforced by the observation that the fitted value of τ_1 drops substantially when a third exponential component is introduced (Table 2).

Incidentally, the standard deviation of the negative initial slope, about 14% of the mean, places an upper limit on the width of any heterogeneity in the value of the rate constant k_T over the population of LH1/RC complexes. Because k_T is inversely proportional to N, the photosynthetic unit size, this result appears to limit any heterogeneity in N over the family of mutants to no more than $\pm 14\%$ of its mean value.

The lack of correlation between the amplitudes of the heterogeneous components in the P* and LH1* decays listed in Table 2 deserves comment. If the PET rate constants are continuously distributed over the RC population, as we assume, biexponential fits imposed on the two sets of decay data are merely approximations. For example, the longer-lived of the two components fitted to the decay of P* in RCs isolated from the wild type or the YY mutant has a lifetime too short to pull the corresponding LH1* decay kinetics away from the transfer-to-trap limit. Thus the biexponential P* decay amplitudes have nothing to do with those fitted to the corresponding LH1* decay, whose longer-lived component lies well outside the transfer-to-trap limit. Although most of the other mutants studied by Jia et al. (1993) have P* decays fitted with second components whose lifetimes imply PET rate constants too small to be in the transfer-to-trap limit, analogous arguments can be made. For example, the 70-ps component fitted to the P* decay of the FT mutant may be viewed as a compromise choice of the fitting routine between the two longer components (of three) fitted to the corresponding LH1* decay. In turn the latter fit simply reflects a better approximation to the underlying continuous distribution of PET rate constants. Thus, we see no reason to expect any correlation of bi- (or tri-) exponential fitting amplitudes of P* and LH1* decays.

As already noted, if the PET rate constant is continuously distributed, there must be an underlying distribution of physical parameters that ultimately depends on distributed structural variables. In the electron transfer theory (Marcus & Sutin, 1985), there are two obvious candidates for the physical parameters. These are the free energy gap, ΔG, and the electron transfer matrix element, V. Possible structural variables that might generate heterogeneous ΔG have been considered (Small et al., 1992; Wang et al., 1993). However, recent experimental evidence (Popov, 1996) points to homogeneous ΔG. Heterogeneity of V seems more likely which is presumed to be a sensitive function of the donor−acceptor separation distance (Moser et al., 1995 and references therein). Additional studies of these issues, both theoretical and experimental (unpublished results), are in progress.

Other multiexponential kinetics have been explained by feedback from secondary electron transfer (Beekman et al., 1994) or a mixed population of open and closed RCs (see Table 1). These phenomena can be ruled out as possible explanations for the LH1* decay data presented here. We tested specifically for the influence of secondary electron transfer using a mutant M250Trp → Phe (M250F) which has a wild-type rate of primary electron transfer and slower secondary electron transfer; $H_A^- \rightarrow Q_A$ is reduced to (880 ps)$^{-1}$ from (200 ps)$^{-1}$ for the wild type (Coleman & Youvan, 1990). Decays of LH1 excited states were the same for this strain as for the wild type (Table 2), thus arguing against the feedback mechanism. In sharp contrast, feedback has been observed in Photosystem II (Schatz et al., 1987).

We can also reject the possibility that the LH1* decay kinetics we observe can be explained by a mixed population of open (PH_AQ_A) and closed ($PH_AQ_A^-$ and/or $P^+H_AQ_A$) RCs within the sample volume. Samples containing a large excess of PMS (an acceptor of electrons from Q_A^-) returned identical multiphasic kinetics as those samples measured with PMS concentrations described in the methods (data not shown). Measurements made on unstirred samples in the absence of PMS (building up a fraction of closed RCs in the sample volume) or in the absence of ascorbate (eventually photooxidizing a fraction of P in the sample volume) resulted in kinetics with new, longer-lived components (≈150 and 170 ps, respectively; data not shown), not simple changes in the amplitudes of those components previously described. These results corroborate previous reports of quenching of LH1* by closed wild-type RCs (Table 1). We attribute the multiexponential behavior with open and reduced RCs to heterogeneity of PET, not to different redox states of the RC.

Why have these results not been observed previously? (1) Single photon counting measurements (with detector-limited response times typically between 50 and 70 ps; Freiberg et al., 1995; 1996; Timpmann et al., 1995) would not be capable of resolving the faster components observed. (2) Transient absorption data with coarse time resolution in the 0 to 20-ps region (Timpmann et al., 1993; Kramer et al., 1995) would be hard-pressed to resolve the faster components observed, and analyses of one-color experiments (Beekman et al., 1994) are complicated by coherence phenomena at early times. (3) The only two-color experiment with sufficient time resolution (Xiao et al., 1994) used global analysis of transient absorption surfaces. Although this technique is usually capable of resolving previously undetected components, the wild-type lifetime that was extracted with 590-nm excitation is intermediate to the ones found in the present study. Superior signal-to-noise achieved with the 1.3-kHz OPA-based transient absorption spectrophotometer and measurements extending the time range of signals observed to more than 600 ps for wild type [and up to 5 ns for mutant strains, compared with only 150 ps in Xiao et al., (1994)] have facilitated the observation of the nonexponentiality of the LH1* decays on the timescale of tens of picoseconds.

CONCLUSIONS

We conclude that LH1* decays are more sensitive indicators of electron transfer heterogeneity than is direct measurement of electron transfer using stimulated or spontaneous emission. The coupled antenna slows and separates the decay components so that they can be resolved more readily. Since excited state decays in LH1 are nonexponential and correlate with the nonexponentiality observed in P* decays in isolated RCs from the same strains, our modeling suggests that the electron transfer heterogeneity observed in isolated RCs is not introduced by the isolation procedure. Observation of both transfer-to-trap and PET kinetic limitations within a single light-harvesting system is unexpected and complicates any discussion of the rate-limiting step of antenna exciton trapping in photosynthesis. It also provides a new method for the analysis of the PET kinetics in photosynthetic systems.

REFERENCES

Beekman, L. M. P., van Mourik, F., Jones, M. R., Visser, H. M., Hunter, C. N., & van Grondelle, R. (1994) *Biochemistry 33*, 3143.

Beekman, L. M. P., Visschers, R. W., Monshouwer, R., Heer-Dawson, M., Mattioli, T. A., McGlynn, P., Hunter, C. N., Robert, B., van Stokkum, I. H. M., van Grondelle, R., & Jones, M. R. (1995) *Biochemistry 34*, 14712.

Bibikova, M., Arlt, Th., Zinth, W., & Oesterhelt, D. (1995) *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. I, p 867, Kluwer Academic Publishers, Netherlands.

Breton, J., Martin, J.-L., Migus, A., Antonetti, A., & Orszag, A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5121.

Bylina, E. J., Ismail, S., & Youvan, D. C. (1986) *Bio/Technology 7*, 69.

Bylina, E. J., Jovine, R. V. M., & Youvan, D. C. (1989) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7226.

Chan, C.-K., Chen, L. X.-Q., DiMagno, T. J., Hanson, D. K., Nance, S. L., Schiffer, M., Norris, J. R., & Fleming, G. R. (1991) *Chem. Phys. Lett. 176*, 366.

Chirino, A. J., Lous, E. J., Huber, M., Allen, J. P., Schenck, C. C., Paddock, M. L., Feher, G., & Rees, D. C. (1994) *Biochemistry 33*, 4584.

Cogdell, R. J., Fyfe, P. K., Barrett, S. J., Prince, S. M., Freer, A. A., Isaacs, N. W., McGlynn, P., & Hunter, C. N. (1996) *Photosynth. Res. 48*, 55.

Coleman, W. J., Bylina, E. J., Aumeier, W., Siegl, J., Eberl, U., Heckmann, R., Ogrodnik, A., Michel-Beyerle, M. E ., & Youvan, D. C. (1990) *Structure and Function of Bacterial Photosynthetic Reaction Centers* (Michel-Beyerle, M. E., Ed.) p 273, Springer-Verlag, New York.

Coleman, W. J., & Youvan, D. C. (1990) *Annu. Rev. Biophys. Chem. 19*, 333.

Du, M., Rosenthal, S. J., Xie, X., DiMagno, T. J., Schmidt, M., Hanson, D. K., Schiffer, M., Norris, J. R., & Fleming, G. R. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 8517.

Ermler, U., Michel, H., & Schiffer, M. (1994) *J. Bioenerg. Biomembr. 26*, 5.

Finkele, U., Lauterwasser, C., Zinth, W., Gray, K. A., & Oesterhelt, D. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7888−7892.

Förster, T (1965) *Modern Quantum Chemistry, Part III* (Sinanoglu, O., Ed.) p 93, Academic Press, New York.

Freiberg, A., Allen, J. P., Williams, J. C., & Woodbury, N. W. (1995) *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. I, p 863, Kluwer Academic Publishers, Netherlands.

Freiberg, A., Allen, J. P., Williams, J. C., & Woodbury, N. W. (1996) *Photosynth. Res. 48*, 309.

Gray, K. A., Farchaus, J. W., Wachtveitl, J., Breton, J., Finkele, U., Lauterwasser, C., Zinth, W., & Oesterhelt, D. (1990) *Reaction Centers of Photosynthetic Bacteria* (Michel-Beyerle, M.-E., Ed.) p 251, Springer-Verlag, New York.

Greenfield, S. R., Seibert, M., Govindjee, & Wasielewski, M. R. (1996) *Chem. Phys. 210*, 279.

Greenfield, S. R., & Wasielewski, M. R. (1995) *Opt. Lett. 20*, 1394.

Hamm, P., Gray, K. A., Oesterhelt, D., Feick, R., Scheer, H., & Zinth, W. (1993) *Biochim. Biophys. Acta 1142*, 99.

Hanson, D. K., L. Baciou, L., Tiede, D. M., Nance, S. L., Schiffer, M., & Sebban, P. (1992) *Biochim. Biophys. Acta 1102*, 260.

Hemenger, R. P., Lakatos-Lindenberg, K., & Pearlstein, R. M. (1974) *J. Chem. Phys. 60*, 3271.

Holzwarth, A. R., & Müller, M. G. (1996) *Biochemistry 35*, 11820.
Hu, X., Ritz, T., Damjanović, A., & Schulten, K. (1997) *J. Phys. Chem. 101*, 3854.
Jia, Y., DiMagno, T. J., Chan, C.-K., Wang, Z., Du, M., Hanson, D. K., Schiffer, M., Norris, J. R., Fleming, G. R., & Popov, M. S. (1993) *J. Phys. Chem. 97*, 13180.
Karrasch, S., Bullough, P. A., & Ghosh, R. (1995) *EMBO J. 14*, 631.
Kirmaier, C., & Holten, D. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 3552.
Kirmaier, C., & Holten, D. (1991) *Biochemistry 30*, 609.
Koepke, J., Hu X., Muenke, C., Schulten, K., & Michel, H. (1996) *Structure 4*, 581.
Kramer, H., Deinum, G., Gardiner, A. T., Cogdell, R. J., Francke, C., Aartsma,T. J., & Amesz, J. (1995) *Biochim. Biophys. Acta 1231*, 33.
Kunkel, T. A., Roberts, J. D., & Zakour, R. A. (1987) *Methods Enzymol. 154*, 367.
Lin, S., Taguchi, A. K., & Woodbury, N. W. (1996) *J. Phys. Chem. 100*, 17067.
Marcus, R. A., & Sutin, N. (1985) *Biochim. Biophys. Acta 811*, 265.
McDermott, G., Prince, S. M., Freer, A. A. Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J., & Isaacs, N. W. (1995) *Nature 374*, 517.
Moser, C. C., Page, C. C., Farid, R.& Dutton, P. L. (1995) *J. Bioenerg. Biomembr. 27*, 263.
Müller, M. G., Drews, G., & Holzwarth, A. R. (1996) *Chem. Phys. Lett. 258*, 194.
Müller, M. G., Griebenow, K., & Holzwarth, A. R. (1992) *Chem. Phys. Lett. 199*, 465.
Nagarajan, V., Parson, W. W., Davis, D., & Schenck, C. C. (1993) *Biochemistry 32*, 12324.
Nagarajan, V., W. W. Parson, W. W., Gaul, D., & Schenck, C. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7888.
Papiz, M. Z., Prince, S. M., Hawthornthwaite-Lawless, A. M., McDermott, G., Freer, A. A., Isaacs, N. W., & Cogdell, R. J. (1996) *Trends Plant Sci. 1*, 198.
Parson, W. W., Nagarajan, V., Gaul, D., Schenck, C. C., Chu, Z.-T., & Warshel, A. (1990) *Structure and Function of Bacterial Photosynthetic Reaction Centers* (Michel-Beyerle, M. E., Ed.) p 239, Springer-Verlag, New York.
Pearlstein, R. M. (1972) *J. Chem. Phys. 56*, 2431.
Pearlstein, R. M. (1982) *Photochem. Photobiol. 35*, 835.
Pearlstein, R. M. (1996a) *Chem. Phys. Lett. 262*, 442.
Pearlstein, R. M. (1996b) *Photosynth. Res. 48*, 75.
Peloquin, J. M., Lin, S., Taguchi, A. K. W., & Woodbury, N. W. (1995) *J. Phys. Chem. 99*, 1349.
Peloquin, J. M., Lin, S., Taguchi, A. K. W., & Woodbury, N. W. (1996) *J. Phys. Chem. 100*, 14228.
Peloquin, J. M., Williams, J. C., Lin, X., Alden, R. G., Taguchi, A. K. W., Allen, J. P., & Woodbury, N. W. (1994) *Biochemistry 33*, 8089.
Popov, M. S. (1996) Ph.D. thesis. University of Chicago.
Prince, R. C., & Youvan, D. C. (1987) *Biochem. Biophys. Acta 980*, 286.
Pullerits, T, & Sundstrom, V. (1996) *Acc. Chem. Res. 29*, 381.
Savage, H., Cyrklaff, M., Montoya, G., Kühlbrandt, W., & Sinning, I. (1996) *Structure 4*, 243.
Schatz, G. H., Brock, H., & Holzwarth, A. R. (1987) *Proc. Natl. Sci. Acad. U.S.A. 84*, 8414.
Simon, R., Priefer, U., & Puhler, A. (1983) *Bio/Technology 1*, 37.
Small, G. J., Hayes, J. M., & Silbey, R. J. (1992) *J. Phys. Chem. 96*, 7499.
Timpmann, K., Zhang, F. G., Freiberg, A., & Sundström, V. (1993) *Biochim. Biophys. Acta 1183*, 185.
Timpmann, K., Freiberg, A., & Sundström, V. (1995) *Chem. Phys. 194*, 275.
Wang, S., Lin, S., Lin, X., Woodbury, N. W., & Allen, J. P. (1994) *Photosynth. Res. 42*, 203.
Wang, Z., Pearlstein, R. M., Jia, Y., Fleming, G. R., & Norris, J. R. (1993) *Chem. Phys. 176*, 421.
Wasielewski, M. R., & Tiede, D. M. (1986) *FEBS Lett. 204*, 368.
Woodbury, N. W., Peloquin, J. M., Alden, R. G., Lin, X., Lin, S., Taguchi, A. K. W., Williams, J. C., & Allen, J. P. (1994) *Biochemistry 33*, 8101.
Xiao, W., Lin, S., Taguchi, A. K. W., & Woodbury, N. W. (1994) *Biochemistry 33*, 8313.
Youvan, D. C ., Ismail, S., & Bylina, E. J. (1985) *Gene 33*, 19.

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