Direct Energy Transfer from the Peripheral LH2 Antenna to the Reaction Center in a Mutant of *Rhodobacter sphaeroides* That Lacks the Core LH1 Antenna†

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ABSTRACT: The light-harvesting apparatus of the photosynthetic bacterium *Rhodobacter sphaeroides* is composed of a peripheral LH2 complex which directs excitation energy to the LH1/reaction center core. The *puf* BA genes encoding the LH1 polypeptides have been deleted, producing a photosynthetically-competent strain which contains LH2 and reaction centers. Time-resolved absorption and fluorescence measurements demonstrate that energy is efficiently transferred from LH2 to the reaction center, despite the absence of LH1. Energy trapping takes place in 55 ± 5 ps at room temperature, compared to the result for the wild-type strain of 60 ± 5 ps. At 77 K, the results for the mutant and wild type are 75 ± 5 and approximately 35 ps, respectively; the slower time in the mutant is attributed to the small differences in antenna/reaction center contacts and relative distances that are bound to exist as a consequence of LH1 and LH2 being assembled from different α-and β-polypeptides. Measurements with closed reaction centers provided new information on the nature of fast energy transfer within the B850 pigments of LH2. We conclude that the absorption band is inhomogeneously broadened, and the fast (~10 ps) lifetime observed in the 847–857-nm region is interpreted as very rapid (1–5 ps) hopping of the excitation energy from high-energy to low-energy pigments within the B850 absorption band. Time-resolved anisotropy studies demonstrate that energy-transfer events within B850 occur on a subpicosecond to picosecond time scale. In respect of internal energy transfer, LH2 therefore behaves similarly to other light-harvesting antennas, and in a wider sense, LH2 can substitute efficiently for LH1 in delivering excitations to the reaction center. This implies some similarity in the three-dimensional arrangement of LH1 and LH2, and notwithstanding this a relatively nonspecific protein–protein interaction between the antenna and the reaction center.

The photosynthetic bacterium *Rhodobacter sphaeroides* has proved to be extremely useful for examining the molecular basis for the processes of harvesting, transfer, and trapping of light energy and its transduction into useful electrochemical energy. The photosynthetic apparatus, which is assembled either under anaerobic conditions in the light or under semiaerobic conditions in the dark, is housed in a highly invaginated intracytoplasmic membrane (Niederman & Gibson, 1978; Cohen-Bazire et al., 1957). The light-driven generation of a transmembrane electrical potential takes place within membrane-bound reaction centers, which are served by antenna light-harvesting pigment–protein complexes. In *Rhodobacter sphaeroides*, this antenna has two components, the proximal LH1 complex which is present in a fixed stoichiometry with the reaction centers and a peripheral LH2 complex, the levels of which vary with growth conditions (light intensity and oxygen tension) (Aagaard & Sistrom, 1972). These complexes are often distinguished by the maxima of the Q_y transitions of their constituent bacteriochlorophyll molecules in the near-infrared, with the LH1 complex having a single maximum at approximately 875 nm (B875) and the LH2 complex having two maxima at approximately 800 and 850 nm (B800–850).

Over the last 10 years, excitation transfer through the *Rb.*[1] *sphaeroides* antenna has been the subject of intensive investigation by picosecond absorption (Sundström et al., 1986; van Grondelle et al., 1987; Bergström et al., 1988; Zhang et al., 1992a) and fluorescence (Borisov et al., 1985; Freiberg et al., 1988; Shimada et al., 1989) spectroscopy. The experimental material has been membranes prepared from the wild type bacterium, which can be represented as LH2+LH1+RC. In addition, it has been possible to study individual antenna complexes by measuring picosecond absorption recovery kinetics of purified LH2 complexes (Bergström et al., 1988) or mutant strains containing LH2, LH1+RC, or LH1 complexes (Hunter et al., 1990). These measurements have revealed that on excitation of the highest energy components of the antenna system, the B800 Bchls of the LH2 complex, excitations arrive at the lowest energy pigments of the LH1 complex [in a two-state model of the LH1 antenna called B896 (Bolt et al., 1981; Borisov et al., 1982; Kramer et al., 1984; Sebban et al., 1985)] with a time constant of 12 ± 1 ps at 77 K (Zhang et al., 1992a). This process of unidirectional energy transfer through the antenna at low temperature involves at least four sequential steps. The first is transfer from B800 to B850 within the LH2 complex, which occurs with a time constant of approximately 2 ps (Bergström et al., 1988; Visscher et al., 1993a,b). This is followed by another fast (~2 ps) transfer from B850 in the LH2 complex to LH1 (Zhang et al., 1992a). There is a slower (~9 ps) transfer from B875 within LH1 to the minor population of red-shifted Bchls (B896 in the two-state model) which has been suggested to act as a focus for excitation energy and forms the immediate donor to the special pair of

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[1] Abbreviations: RC, reaction center; LH, light harvesting; *Rb.*, *Rhodobacter*; *Rs.*, *Rhodospirillum*; P870, reaction center primary donor; ST, singlet–triplet; Bchl, bacteriochlorophyll; PIQ, P^+IQ, $P^+I^-Q^-$, and P^+IQ^-, redox states of the reaction center where P is the primary donor, I is the acceptor bacteriopheophytin, and Q is the primary acceptor quinone.

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bacteriochlorophylls within the reaction center (P870). The final step in which excitations are trapped by the reaction center is a relatively slow process (35 ps at 77 K) in comparison with the 12-ps transfer of energy through the antenna. From a measurement of the detrapping ratio (25%) in the similar bacterium *Rhodospirillum rubrum* at room temperature, it was verified that the trapping step is relatively slow, also approximately 35 ps at room temperature (Timpmann et al., 1993). Thus, it appears that the energy transfer step from the antenna to the reaction center represents the slowest step in the total energy trapping process, largely controlling the rate of the overall process. The slow rate of the trapping step probably arises from distance limitation, with the rate being inversely proportional to the sixth power of the distance between the participating chromophores. It has been proposed that a center to center distance of approximately 3 nm between the antenna molecules coordinating the reaction center and P870 is compatible with the observed rate, and similar distances have been calculated for other species of photosynthetic bacteria (Bergström et al., 1989). One approach to examining the dependence of the rate of trapping on distance and orientation would therefore be to perturb the interaction between LH1 and the reaction center within the core complex by site-directed mutagenesis, so altering the chromophore distance or orientation for the final trapping step. An alternative approach is to examine energy transfer to the reaction center from an antenna other than the *Rb. sphaeroides* LH1 complex, the most obvious candidate for this being the *Rb. sphaeroides* LH2 complex, which forms the sole antenna to the reaction center when assembly of the LH1 complex is prevented.

A mutant of *Rb. sphaeroides* deficient in the LH1 complex (strain RS103) has been described by Meinhardt et al. (1985). The strain was capable of photosynthetic growth at normal rates at high light intensity, but grew more slowly than the wild type at low light intensities. Fluorescence spectroscopy revealed a maximum of fluorescence intensity for the LH1-deficient strain some 10-fold higher than that of the wild type, demonstrating that coupling of the antenna to the reaction center in the mutant strain was considerably less efficient than in the wild type. From a consideration of the effect of attenuation of the intensity of an actinic flash on the amount of reaction center photooxidation in membrane samples, the authors concluded that energy transfer was approximately 25% as efficient in the LH1-deficient strain when compared to the wild type. However, the authors were not able to determine whether direct energy transfer from LH2 to the reaction center was taking place or whether the residual photooxidation was due to a combination of direct excitation of the reaction center by the flash and absorbance by the reaction center of light released from LH2 in a nonspecific manner as fluorescence. Also, it appears that the LH1 α-polypeptide (and also possibly the LH1 β-polypeptide) is present in RS103, which may affect the coupling of LH2 with the reaction center (Kiley et al., 1988).

Recently we have described the construction of a strain of *Rb. sphaeroides* which is deficient in the LH1 complex by virtue of deletion of the RC+LH1 *puf* BALMX operon and complementation with the *puf* LMX genes (Jones et al., 1992). The strain, termed DPF2(pRKEH2), grows relatively slowly at low light intensity, having a doubling time of 8 h compared with 4 h for the wild type. However, it grows more rapidly than a strain which is completely devoid of antenna complexes [strain DD13/G1(pRKEH2); doubling time 12 h], indicating that the LH2 complex can productively transfer energy to the reaction center in the absence of the LH1 complex. Strain DPF2(pRKEH2) differs from previous LH2+RC strains obtained from point mutations in that deletion of the *puf* BA genes provides a strain with the unequivocal absence of LH1 subunits. In this report, we describe a kinetic study of energy transfer from the LH2 complex to both open and closed reaction centers. The results with open reaction centers address the "uniqueness" of core antenna–reaction center interactions. In the case of experiments with closed reaction centers, the dynamics of excitation transfer within a genetically-defined, membrane-bound LH2 complex are presented. The results with closed reaction centers also provide new information on the nature of the relatively fast quenching of antenna excitations by reaction centers with an oxidized primary donor ($P870^+$).

MATERIALS AND METHODS

Construction of a Mutant Which Is Deficient in the LH1 Complex. The construction of the LH1-deficient mutant used has been described in detail previously (Jones et al., 1992). Briefly, a *puf* operon-deletion mutant DPF2 (Hunter et al., 1991) was complemented with an engineered *puf* operon in which the LH1 *puf* BA genes had been excised (Jones et al., 1992). The resulting transconjugant strain, which was called DPF2(pRKEH2), has a spectrum in the near-infrared which is very similar to that exhibited by LH2-only mutants (van Dorssen et al., 1988), but the strain is able to grow photosynthetically, demonstrating the presence of reaction centers.

All measurements were performed on intracytoplasmic membranes prepared from cells of DPF2(pRKEH2) and wild-type strain NCIB 8253, which were grown under semiaerobic conditions in the dark. Membranes were prepared using a French press as described in Clark et al. (1983) and were purified by sucrose step centrifugation. Purified membranes were concentrated by dilution followed by ultracentrifugation and were resuspended in 10 mM Tris (pH 8) and stored at −20 °C prior to use.

Spectroscopy. The dynamics of antenna Bchl excitations were monitored with the picosecond transient absorption (Sundström et al., 1986; van Grondelle et al., 1987; Zhang et al., 1992a) and time-resolved fluorescence (Borisov et al., 1985; Freiberg et al., 1988; Shimada et al., 1989) methods. In the absorption experiments, pigment molecules were excited with a 10-ps pulse in the wavelength range 835–845 nm, and the rise and decay of antenna Bchl excited states were monitored at several wavelengths throughout the B850 absorption band of the LH2 antenna. Kinetics were measured using an apparatus previously described in detail (Zhang et al., 1992a). Briefly, two independently tunable trains of picosecond pulses were generated by parallel pumping of two dye lasers with a mode-locked and frequency-doubled Nd^{3+}-YAG laser operating at a pulse repetition frequency of 82 MHz. The pulse repetition rate of the dye lasers was reduced to 800 kHz by cavity-dumping, and the two pulse trains were synchronized to each other. This resulted in a 10-ps apparatus response function, as given by the cross-correlation pulse of the two dye lasers, measured by sum-frequency generation in a $LiIO_3$ crystal. Deconvolution of measured kinetics with this response function yielded an effective time resolution of ~2 ps. Two types of time-resolved absorption measurements were performed. With the relative polarization of the excitation and probing beams set at the magic angle, isotropic kinetics which reflect only true level kinetics were measured. This measurement is insensitive to absorbance changes caused by changes of sample dichroism. The latter effect was studied in a separate experiment by measurement of the time-resolved

anisotropy, obtained from two independent measurements of the kinetics with the polarization of the probe light parallel or perpendicular to that of the excitation light. In all absorption kinetic curves of this paper (except Figure 6), an induced bleaching is displayed as a positive signal; *i.e.*, the vertical scale of the decays displays $-\Delta A$.

Time-resolved fluorescence was measured by exciting the sample with a ~10 ps pulse at 590 nm in the Q_x absorption band of the antenna Bchls. Fluorescence was detected with a cooled microchannel plate photomultiplier (Hamamatsu R 2809U-05) and time-resolved with the conventional time-correlated single photon counting method. This resulted in an apparatus response function of approximately 50 ps which yielded a 5–10-ps time-resolution when the fluorescence decays measured at several different wavelengths were deconvoluted with the response function and analyzed with a least-squares global-fitting procedure.

Room temperature absorption measurements were performed with the sample in a rotating sample cell of 1-mm optical path length, and low-temperature measurements were performed at 77 K with the help of a liquid nitrogen cryostat. In order to obtain a good glass for the low-temperature measurements, the concentrated solution of membranes was diluted with a buffered glycerol/water mixture (10 mM Tris, pH 8) to yield a sample with a 2/1 glycerol/water volume ratio. Both types of measurements have been described in detail previously (Sundström et al., 1986; van Grondelle et al., 1987). In the fluorescence measurements, which were conducted only at room temperature, the sample was slowly pumped through the measuring cuvette. Kinetics were measured with the reaction centers in a photochemically active (PIQ) or a nonactive (P^+IQ) state. At room temperature, the inactive ("closed") state was accomplished when no additions were made to the sample solution, through the high-pulse repetition rate of the excitation beam which saturates the electron-transfer chain and causes the primary donor to accumulate in the state $P870^+$. Reaction centers in the active ("open") state were obtained by addition of 2 mM sodium ascorbate to the buffer solution containing the sample in order to chemically reduce $P870^+$ between pulses. At low temperature, the photochemically active state was obtained by prereducing the reaction centers with 10 mM sodium dithionite to the state PIQ^-. Upon accepting excitation energy from the antenna, followed by primary charge separation, the state $P^+I^-Q^-$ is formed. This state recombines to the initial PIQ^- state on a time scale which is fast compared to the pulse repetition rate (800 kHz) of the lasers and which maintains the primary charge separation active. Closed reaction center conditions at low temperature were generated in the same way as at room temperature. Measured transient absorption curves were analyzed in terms of a sum of exponentials, and lifetimes and amplitudes were obtained by using a nonlinear least-squares fitting procedure, including deconvolution of the measured kinetics with the cross-correlation apparatus response function. As was already mentioned above, the fluorescence decays were analyzed in a global-fitting procedure.

RESULTS AND DISCUSSION

Excitation Trapping at Room Temperature and 77 K with Open Reaction Centers. The modified antenna–reaction center pigment system of the DPF2(pRKEH2) mutant was constructed in order to determine to what extent energy is transferred from the LH2 antenna to the reaction center, and what the rate of this process is relative to that seen in the RC/LH1 core complex from wild-type *Rb. sphaeroides*. In order to address these questions, we measured the time-dependent decay of antenna excitations, using both time-resolved absorption and fluorescence methods. The absorption measurements, in addition, directly monitor the formation of the charge-separated P^+I^-Q/P^+IQ^- states by virtue of the absorbance changes in the 830–880-nm region associated with the charge separation. We start by discussing the results of the absorption measurements. Figure 1 illustrates the decay of bleaching at 880 nm induced by an excitation pulse at 840 nm. At this wavelength, the observed signal has contributions from ground-state bleaching and stimulated emission from the antenna. On this time scale, both contributions will decay with the same kinetics. The signal is seen to have a pulse-limited rise, followed by a 55 ± 5 ps decay to a much lower level which is constant on the time scale of this measurement. This long-lived bleaching is characteristic of the charge-separated state (Sundström et al., 1986; van Grondelle et al., 1987). Thus, this result demonstrates that trapping and charge separation in DPF2(pRKEH2) occur with a time constant of 55 ± 5 ps at room temperature. For comparison, the corresponding kinetics for excitation trapping in *Rb. sphaeroides* WT are shown in Figure 1B (τ = 60 ± 5 ps). Within experimental error, the observed trapping times for the mutant and the wild-type species are the same. Similar kinetics were observed at other probe wavelengths within the B850 absorption band (results not shown), and the trapping dynamics were always well described by a single-exponential decay.

The trapping time for excitations in membranes of DPF2(pRKEH2) was obtained with an average excitation intensity of $I_{AV} \leq 1$ W cm^{-2} (corresponding to a pulse energy of $I_p \leq 1 \times 10^{13}$ photons cm^{-2} pulse^{-1}). For higher intensities the lifetime is shortened, as a result of singlet–triplet annihilation in the LH antenna; at a five-fold higher excitation intensity, the excited-state lifetime is reduced to approximately 35 ps. At excitation energies lower than 1 W cm^{-2}, no noticeable intensity dependence of the measured lifetime was observed. This effect is also observed with closed reaction centers, but here much lower intensities were required to fully eliminate the annihilation since the excited-state lifetime is almost a factor of 10 longer with closed reaction centers (see below). With the shorter excited-state lifetime in open reaction centers, it is also more difficult to observe some relatively slow quenching at low intensity. Singlet-triplet (ST) annihilation is a result of accumulation of long-lived (5 μs) Bchl and carotenoid triplets (Monger et al., 1977; Valkunas et al., 1991), formed through intersystem crossing (approximately 4% efficiency) from excited singlet antenna Bchl. A triplet state acts as a very efficient quencher of Bchl singlet excitations [at least as efficient as an open reaction center (Valkunas et al., 1991)]. At high average excitation intensities, one or more triplets may be accumulated in each domain of the LH antenna, resulting in an increased quenching of singlet excitations. From a theoretical analysis of the intensity dependence of this quenching, information about the connectivity of the LH Bchl molecules can be obtained (Valkunas et al., 1991). Such an analysis has been performed for the ST quenching data of the DPF2(pRKEH2) mutant and for *Rs. rubrum* and will be published elsewhere. It should be noted that the kinetic measurements presented here, relevant to the transfer and trapping of energy, were all obtained under annihilation-free conditions.

The fluorescence measurements for both DPF2(pRKEH2) and the wild-type species, summarized in Figure 2, confirm the absorption results. The decay of antenna Bchl fluorescence with open reaction centers in DPF2(pRKEH2) (Figure 2A) is largely (>80%) dominated by a 52 ± 5 ps decay component,

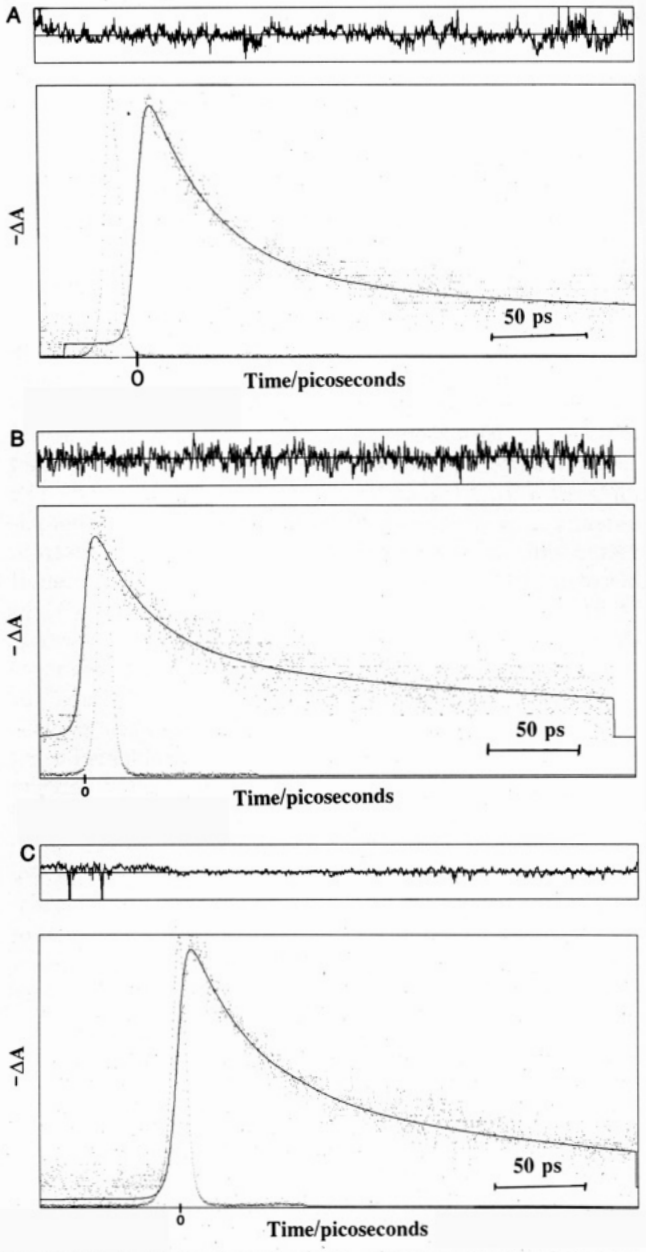

FIGURE 1: (A) Absorption kinetics of DPF2(pRKEH2) at room temperature with open reaction centers, excited at 840 nm and probed at 880 nm. The measurement is performed at low excitation intensity (0.5 W cm^{-2}) where singlet–triplet annihilation is avoided. Fitting parameters: T_1=55 ± 5 ps; T_2 > 1 ns; A_1/A_2 = 4 ± 1. The upper panel displays a plot of residuals. The maximum bleaching corresponds to an absolute absorption change of $\Delta A \approx 5 \times 10^{-4}$. Note that $-\Delta A$ (bleaching) is displayed as a positive signal. (B)Absorption kinetics of *Rb. sphaeroides* WT, measured under the same conditions as for the mutant above in (A). Fitting parameters: T_1=65±5 ps, T_2>1 ns; A_1/A_2 = 3 ± 1. (C) Absorption kinetics of DPF2(pRKEH2) at 77 K with open reaction centers (prereduced Q), excited at 840 nm and probed at 860 nm. Other conditions as in (A). Fitting parameters: T_1 = 70 ± 5 ps; T_2 > 1 ns; A_1/A_2 = 4.5 ± 1.

throughout the measured wavelength interval 860–910 nm, in good agreement with the absorption measurements. Similar results were obtained for *Rb. sphaeroides* WT, (Figure 2B). Less than 5% of the total fluorescence amplitude for both mutant and wild-type species is carried by a much longer >900-ps decay component. In the wild type, the lifetime of this component appears somewhat longer and with less amplitude, as compared with the mutant. Such long lifetimes are characteristic of fluorescence originating from free chlorophylls or from pigment–protein complexes decoupled from the reaction center. The fact that the ratio of the intensities of the fast and slow components was approximately

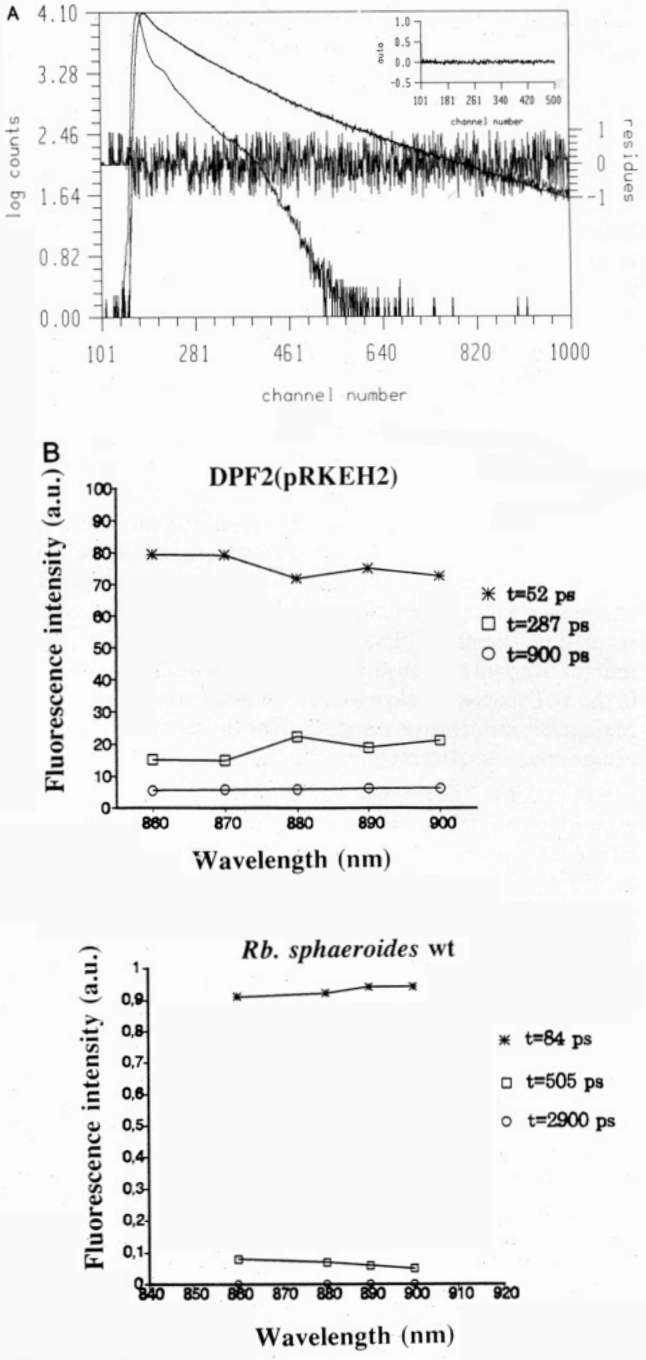

FIGURE 2: Summary of time-resolved fluorescence measurements. (A) Typical fluorescence kinetics, including exponential fit, of DPF2(pRKEH2) with open reaction centers at room temperature. Excitation wavelength 590 nm and detection 890 nm. The time scale is 4.77 ps/channel. Fitting parameters are summarized in (B). (B) Wavelength dependence of relative amplitudes (normalized to 100 at each wavelength) of the different kinetic components, for both mutant and wild type species.

constant throughout the measured wavelength interval favors the latter explanation. There is also a third fluorescence decay component of 300–500 ps time constant and ≤15% amplitude. From experiments performed under closed reaction center ($P870^+$) conditions (discussed below), we conclude that this lifetime reflects the presence of a minor population of closed reaction centers. Under our conditions of high pulse repetition rate (800 kHz) excitation, it is difficult to maintain all reaction centers in their open (reduced) state, with the amount of added sodium ascorbate electron donor used here.

In summary, the combined results of time-resolved absorption and fluorescence measurements on membranes of *Rb. sphaeroides* DPF2(pRKEH2), with open reaction centers,

show that >90% of the excitation energy absorbed by the LH antenna is delivered to the reaction center, where it is trapped with a rate of approximately $(55\ ps)^{-1}$. Both the trapping efficiency and the rate are very similar to those observed in the wild-type species.

These measurements demonstrate directly that LH2 can transfer energy efficiently and rapidly to the reaction centers in this LH1-deficient mutant. It is quite remarkable that the trapping of excitation energy by the reaction center appears to be unaffected by the removal of the LH1 core antenna. Expressed with the help of theoretical random-walk models (Pearlstein, 1982), the trapping rate and efficiency are mainly governed by three different parameters: the magnitude of the relative spectral overlap for Förster energy transfer of the trapping and detrapping processes; the distance from the antenna to the special pair in the reaction center; and the number of antenna molecules effectively delivering excitation energy to the special pair (the coordination around the reaction center). Removal of the LH1 core antenna has the potential to modify all three properties, which might be expected to change the rate and efficiency of trapping. It is all the more surprising, therefore, that the observed trapping time of the mutant at room temperature remains essentially the same as in the WT species. Below we will discuss in some more detail the possible influence on trapping of the three antenna–reaction center coupling parameters.

At first sight, the finding that the LH2 complex can donate excitations to the reaction center rapidly and with high efficiency is surprising in light of previous reports of an LH1-deficient mutant which showed high fluorescence and a low efficiency of excitation trapping (Meinhardt et al., 1985). The difference between strain DPF2(pRKEH2) and the previously reported strain RS103 may lie in the way in which the strains were constructed. In the present case, the LH1 complex is absent by virtue of deletion of the *puf* BA genes (Jones et al., 1992), with the result that the LH1 polypeptides are not synthesized. In the case of strain RS103, the LH1 complex was absent by virtue of point mutation(s) which had an adverse effect on assembly of the LH1 antenna, leading to turnover of LH1 polypeptides in the membrane (Kiley et al., 1988). This raises the possibility that close interaction of the LH2 complex with the reaction center could be prevented in strain RS103 by the presence of the LH1 α- and/or β-polypeptide. By adopting an approach which involves the deletion of the genes which code for unwanted polypeptides, uncertain effects of this sort can be avoided.

It would be reasonable to assume an optimized design of LH1–reaction center contacts and interchromophore distances, which could not be easily reproduced for the peripheral LH2 antenna. The fact that the rate of trapping is virtually the same in DPF2(pRKEH2) and the WT species thus suggests that the part of the core antenna surrounding the reaction center is not highly specialized, with finely tuned antenna–reaction center distances and a species-specific number of antenna molecules dedicated to each reaction center. Rather, it appears that when antenna polypeptides pack around the reaction center a suitable organization is formed more or less spontaneously; the exact organization of the antenna polypeptides around the reaction center is not known. A 6-fold symmetry for the coordination of antenna complexes to the reaction center has often been assumed (Stark et al., 1984). However, the noncircular symmetry of the reaction center itself in the plane of the photosynthetic membrane (Deisenhofer et al., 1985) suggests that the number of antenna molecules which can efficiently transfer energy to the reaction center could be rather small. From theoretical considerations, it has been suggested (Pearlstein, 1992) that the reaction center could even be monocoordinated, with a single antenna Bchl species acting as a conduit for the delivery of excitations. From the present results for trapping in DPF2(pRKEH2), it is not possible to discriminate between the two coordination models. With the already noticed similarity of LH2 and LH1 polypeptides (Brunisholz & Zuber, 1992), both arrangements could probably provide a similar relatively nonspecific reaction center–antenna contact.

The sensitivity of trapping to the spectral changes associated with the substitution of LH2 for LH1 was estimated by calculating the Förster spectral overlap integrals for trapping and detrapping. The results are visualized as a graphical comparison between the mutant and the wild-type species in Figure 3, at both room temperature and 77 K. The actual calculated overlaps are also given below as ratios of overlap integrals with LH2 and LH1 involved, respectively. The antenna fluorescence spectrum of the mutant was approximated with the previously measured fluorescence spectrum of isolated LH2 or the spectrum of a similar LH2-only mutant of *Rb. sphaeroides* (Vos et al., 1988). Independent checks show that this is a good approximation. It should, however, be mentioned that a quantitative comparison between measured trapping rates and Förster spectral overlaps for antenna→reaction center energy transfer is associated with several difficulties. For instance, there is a problem of using correct absorption and fluorescence spectra for the antenna and special pair; the absorption spectrum of P as measured in isolated reaction centers is known to be shifted relative to the *in vivo* spectrum (Zhang et al., 1992b). However, for *Rb. sphaeroides*, this appears not to be a serious problem (Duysens et al., 1956). Further, the influence of relative orientations of antenna and P molecules and the influence of pigment heterogeneity etc. are difficult to assess. Despite these difficulties, we believe that a qualitative comparison of spectral overlaps for the DPF2(pRKEH2) mutant and the wild type is helpful in order to understand the trapping process. Moreover, these difficulties are quite general and exist for both mutant and wild type, thus, as long as only a relative comparison is required, the errors should be limited.

From the absorption and fluorescence spectra of Figure 3, it is seen that the spectral overlap at room temperature for LH2→reaction center energy transfer is very similar to that of LH1→reaction center transfer (Figure 3A), which is confirmed by the Förster overlap integral calculation yielding overlap(LH2→RC)/overlap(LH1→RC) = 0.97. This in combination with the fact that there is a much smaller overlap for reaction center→antenna detrapping (Figure 3C) for the mutant [overlap(RC→LH2)/overlap(RC→LH1) = 0.13] makes the experimental observations understandable. At 77 K, the situation is somewhat different, with the measured trapping time of DPF2(pRKEH2) (70 ± 5 ps, Figure 1C) being a factor of 2 longer than in the wild type (35 ps), at the same temperature (Visscher et al., 1989). The spectra of Figure 3B suggest that the trapping at 77 K should be more efficient in the mutant than in the wild-type species [overlap(LH2→RC)/overlap(LH1→RC) = 2.3]. This is clearly not in agreement with the experimental findings. However, in saying this, it should also be noticed that this discrepancy is by no means remarkable, considering the sixth-power dependence on distance and spectral overlap factors (through the Förster radius R_0) of the energy-transfer step from antenna to primary donor. It is not larger than the natural variation provided by the two slightly different LH1 antennae of *Rb. sphaeroides* and *Rs. rubrum*, where for the latter species the trapping time at 77 K was observed to be

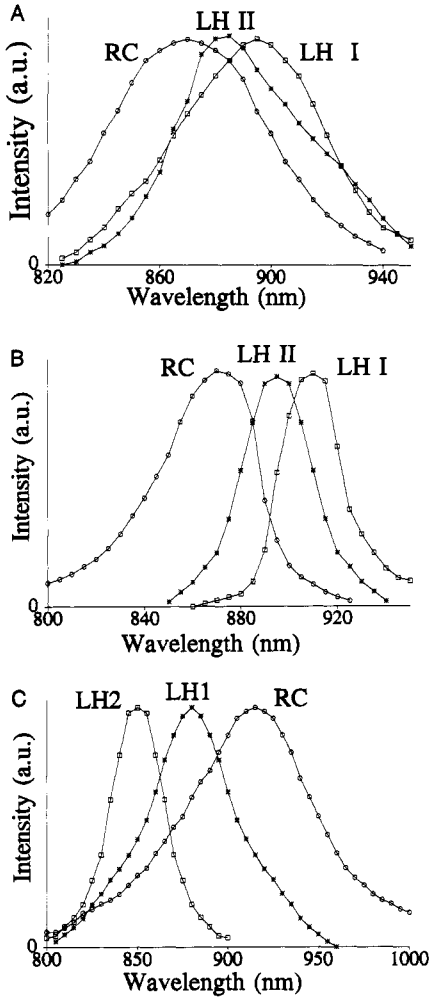

FIGURE 3: Comparison of Förster spectral overlaps for trapping and detrapping involving the LH2 or the LH1 antenna. The spectra for these calculations were obtained from the literature or measured by us. Fluorescence and absorption spectra of membrane bound B850: van Dorssen et al., 1988. Fluorescence spectrum of LH1: Sebban et al., 1985; K. Timpmann, unpublished results. Absorption spectrum of LH1: van Dorssen et al., 1988, and measured by us on LH1-only mutant of *Rb. sphaeroides*. RC absorption and fluorescence spectra: Lockhart et al., 1988; Lösche et al., 1987; Woodbury & Parson, 1984; Lockhart & Boxer, 1988b. See the text for actual values of overlap integral ratios. (A) Förster overlap for trapping at room temperature, involving the fluorescence spectrum of LH2 (*) or LH1 (□) and the absorption spectrum of P870 (○). For clarity, the 800-nm band of the RC spectrum has been removed in this plot. (B) Förster overlap for trapping at 77 K, involving the fluorescence spectrum of LH2 (*) or LH1 (□) and the absorption spectrum of P870 (○). As in (A), the 800-nm RC band has been removed. (C) Förster overlap for detrapping at 77 K, involving the absorption spectrum of LH2 (*) or LH1 (□) and the fluorescence spectrum of P870 (○).

70 ps (Visscher at al., 1989). Hence, the different trapping times of the mutant and wild-type species at 77 K can easily be explained by the small differences in antenna/RC contacts and distances that are bound to exist as a consequence of LH1 and LH2 being assembled from nonidentical α- and β-polypeptides. Here it should also be mentioned that in order to maintain an active primary charge separation at 77 K the trapping was measured with the reaction center initially in the reduced state PIQ^- (by addition of 10 mM sodium dithionite). The $P^+I^-Q^-$ state formed after primary charge separation recombines on a time scale fast compared with the pulse repetition rate of the dye lasers (800 kHz). This restores the reaction center to the active PIQ^- state before the next excitation pulse. It is known from measurements on other species at room temperature (Zhang et al., 1992b; Borisov et al., 1985) that charge separation is somewhat slower when the quinone electron acceptor is in the reduced state. However, since both the mutant and the wild type were measured under similar conditions, we do not expect the comparison between the two to be affected by the prereduction of the reaction center. We conclude this section on trapping in *Rb. sphaeroides* DPF2(pRKEH2) by noting that the observed trapping behavior can largely be explained with spectral overlap considerations. This suggests either that specific antenna–reaction center contacts involving highly specialized arrangements are not important in this species in order to obtain efficient antenna→reaction center energy transfer or that the similarity between LH2 and LH1 is such that specific contacts with the reaction center are preserved when LH2 is substituted for LH1.

Closed Reaction Centers at Room Temperature and 77 K. (A) Quenching of Antenna Excitations by $P870^+$. Closed reaction centers ($P870^+$) are relatively efficient quenchers of antenna excitations, as judged by the approximately 200-ps excited-state lifetime of several purple bacterial species (Sundström et al., 1986; van Grondelle et al., 1987; Borisov et al., 1985), but the mechanism of quenching is poorly understood. Substituting the LH2 complex for the LH1 core antenna provides an opportunity to gain new insight into the mechanism of antenna excitation quenching by $P870^+$. Figure 4 illustrates the picosecond absorption results of membranes of DPF2(pRKEH2) with closed reaction centers at room temperature and 77 K. At a sufficiently low excitation intensity ($\leq$0.1 W cm^{-2} average intensity and $\leq 1 \times 10^{12}$ photons cm^{-2} $pulse^{-1}$ pulse energy), the singlet–triplet quenching appears to be eliminated, and close to single-exponential decays with 330 ± 30 ps time constants are observed both at room temperature (Figure 4A) and at 77 K (Figure 4B). Under the conditions used, the excitation density where annihilation stops corresponds to approximately 1000–1500 pigment molecules/photon. This suggests a quite large domain size of the LH2 antenna of this mutant. A more detailed quantitative analysis of this intensity dependence will be presented elsewhere. For comparison, kinetics measured with 5-fold higher excitation energy are also shown, (Figure 4C). The higher energy causes singlet–triplet annihilation which leads to nonexponential decay kinetics, approximately described by a 30–50-ps and a 330-ps lifetime. Very similar kinetics were obtained from time-resolved fluorescence measurements (not shown). The 330-ps lifetime due to excitation quenching by the closed reaction centers should be contrasted with the 200-ps lifetime observed for the wild-type species under similar conditions.

With the LH2 and LH1 antenna fluorescence spectra and the P^+IQ^- minus PIQ difference absorption spectrum of Figure 5, a possible explanation to the closed reaction center ($P870^+$) antenna excitation quenching may be suggested. The ΔA spectrum for charge separation shows that $P870^+$ develops an absorption band at wavelengths >900 nm, where initially P870 has very little absorption. This $P870^+$ absorption has good spectral overlap with the fluorescence from LH1, but less overlap with the LH2 fluorescence. This directly demonstrates that there are possibilities for energy transfer from the LH1 antenna to $P870^+$. Since $P870^+$ cannot transfer electrons, the energy is probably dissipated as heat through a radiationless process. The smaller spectral overlap for LH2→$P870^+$ energy transfer also explains why DPF2(pRKEH2) with closed reaction centers has a considerably longer antenna excited-state lifetime than the wild-type species. The broad $P870^+$ absorption band also explains why the quenching time with closed reaction centers is virtually temperature-independent

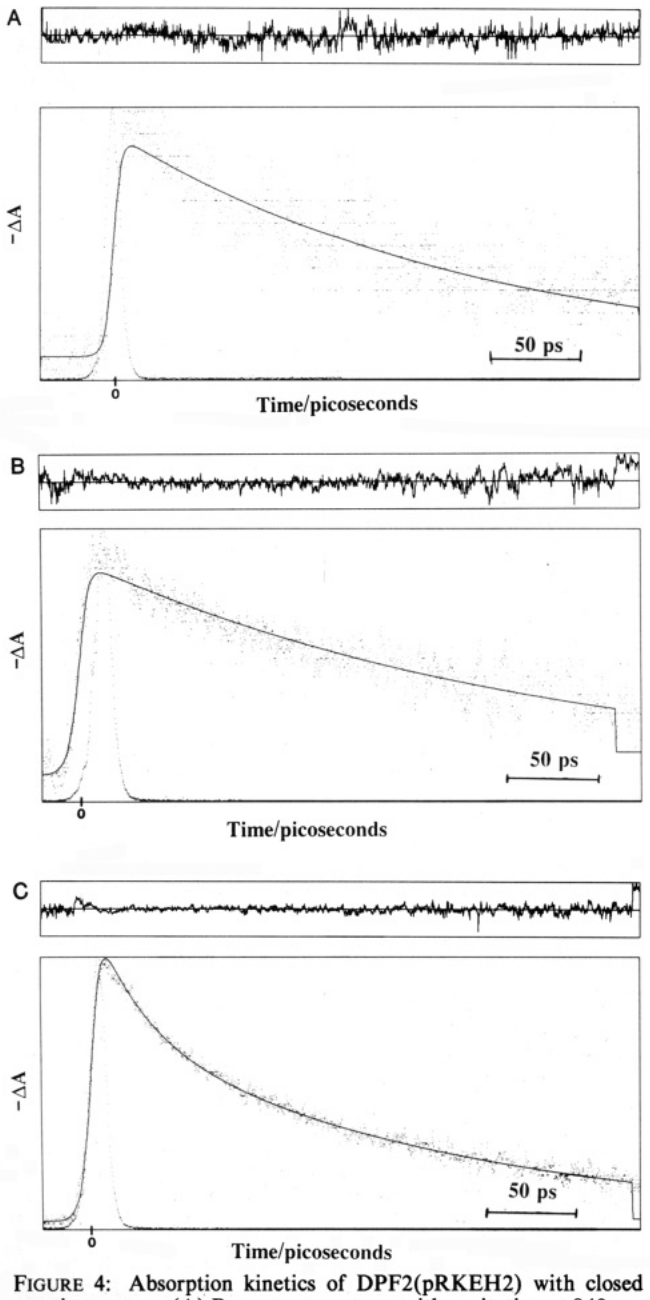

FIGURE 4: Absorption kinetics of DPF2(pRKEH2) with closed reaction centers. (A) Room temperature, with excitation at 840 nm and probing at 880 nm; (B) 77 K, with excitation at 840 nm and probing at 860 nm; and (C) high excitation intensity yielding singlet–triplet annihilation and nonexponential excited-state decay; wavelengths of excitation and probing were the same as in (B). The traces in (A) and (B) are measured at low excitation intensity where the ST quenching is largely avoided, and close to single-exponential decays with 330 ± 30 ps lifetime are obtained (see the text for details). Bleaching, $-\Delta A$, is displayed as a positive signal.

in all studied species of purple bacteria (Sundström et al., 1986; van Grondelle et al., 1987; Freiberg et al., 1988; Shimada et al., 1989), including the present DPF2(pRKEH2) mutant (data not shown).

(B) Fast Energy Transfer within the B850 Pigment. Picosecond absorption measurements with closed reaction centers have in the past been helpful in establishing the time scales and extent of energy migration and redistribution within light-harvesting antenna systems. Measurements with magic angle (MA = 54.7°) relative polarization of the pump and probe beams monitor energy transfer between energetically different LH molecules, but are insensitive to absorbance changes due to changes in dichroism (depolarization). The time-resolved anisotropy, $r(t)$, is, on the other hand, sensitive

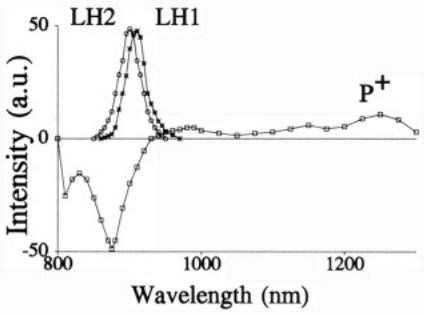

FIGURE 5: Fluorescence spectra of LH2 and LH1 at 77 K and absorption difference spectrum for primary charge separation (Clayton, 1980), for estimation of Förster spectral overlap of energy transfer from the antenna to the oxidized primary donor $P870^+$.

to depolarization when the involved chromophores are energetically (spectrally) equivalent. A measurement of $r(t)$ can therefore be a means of resolving energy transfer between identical or similar LH molecules, for which the MA absorbance changes would be too small to be resolved. For clarity, it should also be pointed out that energy transfer in a system of spectrally disordered pigment molecules having different but strongly overlapping absorption spectra (i.e., an inhomogeneously broadened spectrum) would give rise to similar lifetimes in MA and anisotropy measurements. The LH2+RC mutant under investigation here provided an opportunity to gain more detailed knowledge of energy migration within a membrane-bound LH2 antenna complex in the absence of LH1.

In order to assess the degree of spectral inhomogeneity within the B850 absorption band of the DPF2(pRKEH2) mutant, we excited the antenna with a picosecond pulse at 835–845 nm at 77 K with closed reaction centers and monitored the rise and decay of the antenna excitations at several different wavelengths in the range 835–880 nm. The recorded kinetics are consistent with the absorbance difference spectra generally observed for Bchl pigments (Nuys et al., 1986; van Grondelle et al., 1987), displaying an intense bleaching at wavelengths longer than the absorption maximum and excited-state absorption at shorter wavelengths. An isosbestic point is observed at 852 nm, close to the absorption maximum. Figure 6 shows the recorded kinetics close to this isosbestic wavelength. In terms of a sum of exponentials, the kinetics at all wavelengths can be described with three lifetimes: one short (~10 ps), one intermediate ~30 ps, and one much longer (approximately 350 ps), the origin of which was discussed in the previous paragraph. The approximately 30-ps lifetime is most likely due to some residual ST quenching since these measurements had to be performed at an intensity where annihilation is not completely eliminated. The very short lifetime can most clearly be observed within an approximately 10-nm wavelength region around the isosbestic wavelength (852 nm) of the 350-ps component, (see Figure 6). The fast (~10 ps) lifetime is reminiscent of similarly fast decays observed earlier in both LH1 (van Grondelle et al., 1987; Pullerits et al., 1993) and LH2 (Bergström et al., 1986, 1988) antennas, and we assign it to fast energy transfer from high-energy to low-energy pigments within the inhomogeneously broadened B850 absorption band. Using a master-equation approach (Pullerits et al., 1992) to describe the energy transfer between Bchl 850 molecules having an inhomogeneously broadened absorption band, the ~10-ps lifetime is concluded to correspond to fast 1–5-ps hopping of the excitation energy between small clusters of LH Bchl molecules. At room temperature, this feature is not observed, probably as a result of too fast hopping in comparison with the time resolution, and/or too small absorbance change because of broad spectra at the higher

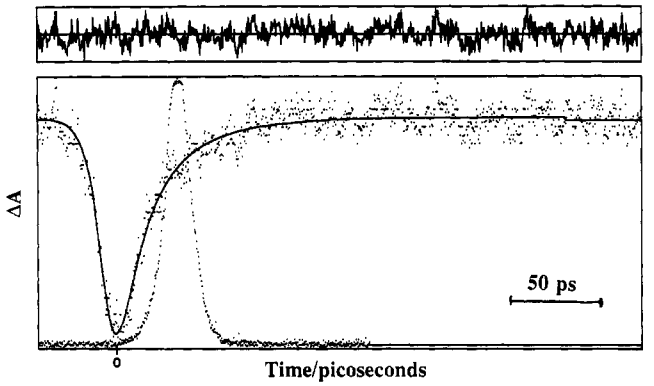

FIGURE 6: Absorption kinetics of DPF2(pRKEH2) at 77 K close to the isosbestic wavelength 852 nm of the main 330-ps decay component of closed reaction centers. Fitting parameters are $T_1 = 10.5 \pm 2$ ps, $T_2 = 27 \pm 3$ ps, $T_3 = 385 \pm 15$ ps, and $A_1/A_2/A_3 = 1/1.5/0.05$. Note that bleaching is displayed as a negative signal, which is opposite to the convention used in the other kinetic traces of this paper.

temperature. Similar results have also been obtained for the LH1 antenna of *Rs. rubrum*, based both on picosecond absorption and on fluorescence measurements (Pullerits et al., 1993). Results of this kind demonstrate that there is fast energy transfer from blue-absorbing to red-absorbing pigments within the LH antenna. This has often been described in terms of a two-state model: B875/B896 for LH1 (van Grondelle et al., 1987; Zhang et al., 1992a) and B850/B870 for LH2 (van Grondelle et al., 1987; Hunter et al., 1990). At temperatures higher than 77 K, this is in most cases a reasonably good description, because the largest spectral inhomogeneities are on the order of the thermal energy (kT). At lower temperatures, fluorescence (Timpmann et al., 1991) and absorption (Visscher et al., 1992; unpublished results) kinetics become strongly nonexponential, and the two-state model does not adequately describe the energy-transfer kinetics. A multistate model arising from inhomogeneous broadening of the LH spectra appears to be a better description (Freiberg et al., 1988; Pullerits & Freiberg, 1992). Excitation wavelength dependent LH fluorescence spectra measured at 4 K were given a similar interpretation (van Mourik et al., 1992).

In the case of a weakly coupled antenna system where energy transfer occurs via Förster hopping, measurements of time-resolved anisotropy probe the extent and time dependence of energy transfer between spectroscopically identical or similar antenna molecules. Alternatively, time-dependent depolarization could also reflect relaxation between differently polarized exciton states in a strongly coupled pigment system. The result of such an anisotropy measurement on DPF2-(pRKEH2) probed at 860 nm with excitation at 840 nm is displayed in Figure 7. It is seen that already within the time resolution of the experiment (~10 ps) the bleaching signal at 860 nm is strongly depolarized; the anisotropy extrapolated to time zero, $r(0)$, has already decayed to a low value (~0.05) and does not decay significantly during the excited-state lifetime of the B850 pigment. This shows that very fast depolarizing energy transfer or possibly exciton-state relaxation occurs within B850 on a subpicosecond to picosecond time scale; the constant value of the long-time anisotropy, $r(\infty) \approx 0.05$, is consistent with complete depolarization in a plane. Similar fast depolarizations have earlier been observed in most Bchl antenna pigment systems (Sundström et al., 1986; van Grondelle et al., 1987). Within experimental error, identical results were obtained at several other probe wavelengths within the interval 835–880 nm. The results of this section clearly show that there is at least one energy-transfer or relaxation process on the few picoseconds to subpicosecond

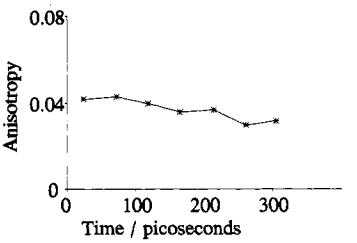

FIGURE 7: Time-resolved absorption anisotropy of DPF2(pRKEH2) with open RC at 77 K, constructed from parallel and perpendicularly polarized kinetics, excited at 840 nm and probed at 860 nm.

time scale in the LH2 antenna of the DPF2(pRKEH2) mutant. In that sense, this antenna system appears to behave very similarly to several other purple bacterial and other LH antennas. However, with the presently used time resolution, we cannot directly time-resolve these processes. In very recent measurements on several purple bacterial LH systems, using subpicosecond and femtosecond pulses, these processes were for the first time directly resolved and studied in detail. The results of this work will be presented elsewhere (Visscher et al., 1993a,b). Here it suffices to say that the more detailed measurements confirm the conclusions drawn above that energy equilibration over spectrally nonequivalent and differently oriented LH Bchl molecules occurs on the time scale ~0.2–1.0 ps, within the B800, B850, and B875 pigments of the LH2 and LH1 antennas. Whether these processes are best described as Förster transfer or if interexciton-state relaxation in a strongly coupled system also contributes to the observed dynamics will be discussed in forthcoming publications (Visscher et al., 1993b). It is interesting to notice that recent hole-burning results on LH2 and LH1 of *Rb. sphaeroides* (Reddy et al., 1991, 1992) were interpreted as fast (approximately 100 fs) relaxation from higher- to lower-lying exciton states of the B850 and B875 pigments.

Antenna Organization in Light-Harvesting Mutants. The data presented in this report demonstrate for the first time that excitation energy can be transferred to the reaction center from the peripheral LH2 on a rapid time scale comparable to that seen for native RC/LH1 core complexes. This indicates that the association formed between LH2 and the reaction center in the absence of the LH1 α-and β-polypeptides is very nearly as intimate as the interaction seen between reaction centers and the LH1 complex. Such minor differences that are seen between the trapping rate in *Rb. sphaeroides* RC/LH1 and RC/LH2, and the temperature dependence of this rate, can easily be accounted for by consideration of two factors. First, the spectral overlap between the emission spectrum of the antenna complex and the absorption spectrum of the P870 differs between the two systems, especially at 77 K. Second, the rate of trapping is proportional to the sixth power of the distance between the participating chromophores, and hence is extremely sensitive to the distance between P870 and the Bchl of closest approach to the reaction center in LH2. The similarity between the rates of trapping in RC/LH1 and RC/LH2 complexes raises interesting questions concerning the specificity of the interaction between the reaction center and the LH1 complex polypeptides and the control of photosystem assembly. The order of assembly of the photosynthetic apparatus in *Rb. sphaeroides* clearly starts with the formation of the RC/LH1 core. There would appear to be no overriding recognition events governing the preferential association of LH1 and reaction center complexes, as evidenced by the fact that LH2 can form an intimate association with the reaction center, and so the arrangements of LH1/RC complexes surrounded by lakes of LH2 proposed initially by Monger

and Parson (1977) appear to be a consequence of temporal events during gene expression and assembly. Since the homologies between LH1 and LH2 polypeptides have already been noted (Brunisholz & Zuber, 1992) it is tempting to suggest that the architecture and organization of the two complexes in the membrane may be similar, a point which may be addressed by progress on the crystallography of these complexes (Nunn et al., 1992; Papiz et al., 1989). Thus, LH2 would appear to be able to substitute for LH1 as a rapid donor of excitation energy to the reaction center.

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