Pathways of energy flow through the light-harvesting antenna of the photosynthetic purple bacterium *Rhodobacter sphaeroides*

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ABSTRACT Using low intensity picosecond absorption spectroscopy with independently tunable excitation and probing infrared pulses, we have studied the pathways of energy transport through the light-harvesting antenna pigments of the photosynthetic purple bacterium *Rhodobacter sphaeroides*. From the observed excited-state rise time of the red-most pigment B896 as a function of excitation wavelength it is concluded that the B850 pigment of LH2 is spectrally heterogeneous. For excitations originating in the B850 pigment this results in a fast channel (9 ps) that is mainly excited in the peak of the B850 absorption band, and a slow channel (35 ps) that is predominantly excited at ~840 nm. Upon excitation of B800, more than 90% of the excitations follow the fast path. From the observed kinetics it is concluded that the majority of the LH2 → LH1 energy transfer takes place within at most a few picoseconds. The rate-limiting step in the whole energy transfer sequence appears to be the B896 → reaction center transfer. The origin of the B850 heterogeneity and the slow 35-ps component is at the moment unclear. Possibly it represents a highly extended form of LH2 in which transfer to LH1 takes a relatively long time, due to a large number of transfer steps.

INTRODUCTION

Knowledge of the dynamics of the photosynthetic primary processes is essential for our understanding of the organization and function of the photosynthetic pigment systems. These primary events of photosynthesis have been studied in a number of different pigment systems, including green plants, algae, and green and purple photosynthetic bacteria. Photosynthetic purple bacteria are attractive to study in this respect because they contain only one photosystem, and various pigment-protein complexes can be isolated to a high degree of purification. Our understanding of pigment organization and primary events of photosynthesis has greatly increased during the last ten years. Starting with the view of the light-harvesting antenna being a homogeneous lattice of pigment molecules and the energy transport through the antenna being a purely random diffusive process (1, 2), today a much more complex picture has evolved. The antenna is currently described as a heterogeneous pigment system consisting of several spectrally distinct pigment-protein complexes (3, 4) efficiently coupled to each other, and the energy transport is characterized by different time scales related to different levels of pigment organization.

Spectroscopic and biochemical methods have shown that the light-harvesting antenna of *Rb. sphaeroides* consists of two different major pigment-protein complexes, LH1 and LH2, respectively. Each pigment system is built of α and β polypeptides that bind the bacteriochlorophyll and carotenoid molecules. Various models exist for the aggregation of the individual polypeptides to form a functional antenna. It appears likely that (αβ) heterodimers form a fundamental unit which is then further aggregated into larger structures $(\alpha\beta)_2$, $(\alpha\beta)_3$, etc. (5, 6). Picosecond polarized absorption measurements have indicated that in the smallest unit $(\alpha\beta)_2$ the excited state is effectively depolarized within 1 ps (8, 9, 21). Energy transfer between $(\alpha\beta)_2$ units may then proceed on a 1–10 ps time scale. In addition to the LH1/LH2 heterogeneity of the light-harvesting antenna a more subtle heterogeneity exists. Picosecond absorption (7–11) and fluorescence (12–17) measurements as well as steady-state fluorescence polarization measurements (18), have shown that both LH1 and LH2 are heterogeneous. Thus, LH1 appears to contain a minor antenna component, called B896, at lower energy than the main LH1 antenna. Picosecond absorption measurements have shown that at room temperature there is a rapid (≈30 ps) equilibration of the excitation energy between the low lying component and the main antenna (8), and that at 77K there is a unidirectional transfer of energy from the main LH1 antenna to B896, within ~10–20 ps. The function of this low lying B896 antenna component has been suggested to be that of focussing the energy to the vicinity of the reaction center and to provide a special entry to the reaction center (7, 9, 19, 20). The rate of energy transfer from B896 to the special pair (P) of the reaction center was measured in the temperature range 177–77K and found to be $(35\ \text{ps})^{-1}$ independent of temperature within this range. Probably, this rate is not much different at room temperature. The low

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value is thought to reflect the relatively large distance of closest approach between the Bchl896 and P (19).

Picosecond absorption (9, 11) as well as fluorescence measurements (16) also suggested a heterogeneity of the LH2 antenna, but the origin of this heterogeneity and coupling of LH2 to the LH1 antenna remained unclear. A closer examination of this is one of the objectives of this work. Another objective is to study the pathways of energy transport through the light-harvesting antenna. This requires independent excitation and probing of the excited state rise and decay of the coupled pigments, which can be achieved by measuring time-resolved picosecond absorption or fluorescence spectra. In earlier experiments, fluorescence decays were measured at several wavelengths, but with only one or a few excitation wavelengths (12, 15, 16), while picosecond absorption measurements have been performed using the single-wavelength technique (8, 9, 21–23), where excitation and probe pulses are of the same wavelength.

The results of this work show that a major part of the optical energy absorbed by the light-harvesting antenna reaches the vicinity of the reaction center within ~10 ps, and that only a minor part of the energy follows another slower (≈35 ps) route. The results further suggest that the LH2 and LH1 antennas are efficiently coupled to each other and transfer between them occurs within only a few picoseconds. These results, combined with the previous knowledge that energy transfer from the antenna to the special pair of the reaction center, B896 → P870, is slow (35 ps) (19, 20), show that the final energy transfer step to the reaction center is much slower than the diffusion of the major part of the energy through the antenna.

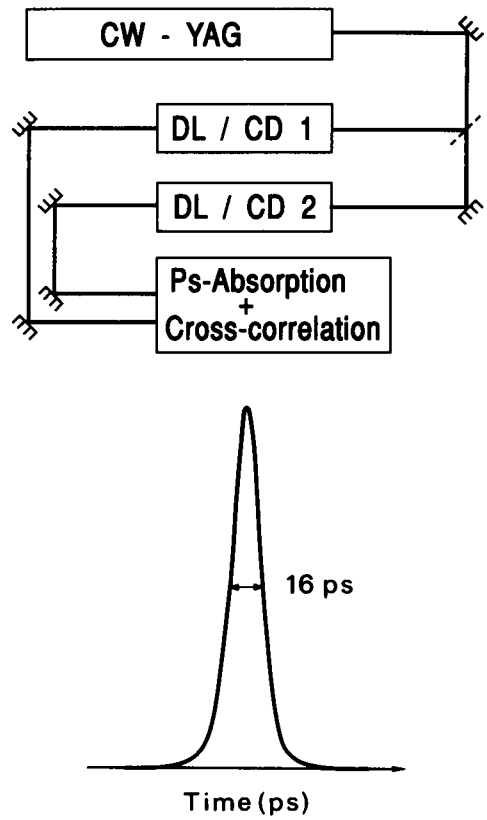

FIGURE 1 (*A*) Schematic representation of the experimental setup. (*B*) Cross-correlation pulse, representing the apparatus response function.

EXPERIMENTAL

In order to obtain the desired information about the energy migration dynamics through the light-harvesting antenna of *Rb. sphaeroides*, we have used the picosecond absorption technique with independently tunable wavelength of excitation and analyzing pulses. This gives us the possibility to measure absorption and excitation spectra with picosecond time resolution. In this work we represent the results of the measurements as excitation spectra of decay or rise-time components.

The absorption kinetics was measured using a dual sync-pumped cavity-dumped single-jet dye laser system (Spectra-Physics, Mountain View, CA, USA) as depicted in Fig. 1. In this setup the two dye lasers are pumped by the same mode-locked CW Nd-YAG laser, and independently tunable over the wavelength range 800–920 nm. The time resolution in this absorption experiment is determined by the width of the cross-correlation pulse of the two dye laser pulses. Such a cross-correlation pulse, measured by sum-frequency generation in a $LiIO_3$ crystal, is shown in Fig. 1. It is seen that the cross-correlation pulse is not much broader than the auto-correlation pulse of each individual dye laser pulse, which shows that the synchronization and jitter between the two dye laser pulse trains is within ~2 ps.

In order to measure annihilation-free excited-state lifetimes of Bchl molecules within a pigment-protein complex, excitation pulses with sufficiently low intensity must be used. This was accomplished by using pulse excitation intensities of $\sim 10^{12}$ photons cm^{-2}, which results in less than 1/1000th of the bacteriochlorophyll molecules being excited. This effectively prevents singlet–singlet annihilation in the present pigment system. Because high repetition rate pulse trains are used, the effect of singlet-triplet annihilation (26) of the Bchl excitations should also be considered. As a consequence of the long triplet lifetimes ($Car^T \approx 5$ μs) (27), buildup of carotenoid triplets may occur, which will lead to singlet-triplet annihilation of the Bchl singlet excitons due to the high mobility of the singlet excitons. This problem is avoided in this work where we use pulse repetition rates lower than, or equal to, 800 kHz, and a maximum excitation degree of 10^{-3} photons/Bchl. We can estimate the influence of carotenoid triplet states on the antenna exciton lifetime by assuming a carotenoid triplet yield of ~4% (28). With the excitation degree given above this results in less than 0.01 carotenoid triplet per RC. Assuming further that the carotenoid triplets are quenching the antenna singlet excitations at least as efficiently as open reaction centers, results in ~1% reduction of the antenna singlet state lifetime. Hence, under the conditions used here the distortion of measured lifetimes by singlet-triplet annihilation is small.

We have previously shown that the decay of light-harvesting antenna excitations measured at room temperature, reflects the equilibration of energy between different spectral forms within the antenna (8). This

makes it difficult to observe the flow of energy between the spectrally different pigments of the antenna, and measure the associated rates of transfer. In addition, the broad and extensively overlapping absorption bands will result in poor spectral resolution at room temperature. This problem can largely be overcome if the excited state kinetics is measured at low temperature (77K). At 77K the excitation transfer proceeds efficiently, is effectively unidirectional, and the spectral resolution is much improved.

In this work we have measured the rise and decay of absorbance changes reflecting the Bchl excited states at 77K for several analyzing wavelengths, as a function of excitation wavelength. The chosen excitation and analyzing wavelengths are summarized in Fig. 2. 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 measured kinetics with the measured cross-correlation pulse (see Fig. 1). Error limits of the given lifetimes represent the standard deviations of typically ten independent measurements. All measurements of this work are performed on chromatophores of *Rb. sphaeroides*. The samples were prepared as described previously (9), and all measurements were performed with photochemically inactive, "closed," reaction centers, where P is in the oxidized state (P^+). When not stated otherwise, all measurements were performed with magic angle (54.7°) relative polarization of the excitation and probing beams. This results in a measurement of the (de)population kinetics of the various pigments involved, with no contribution from anisotropy changes due to transfer between identical, but differently oriented chromophores.

RESULTS

Overall transfer time of energy transport through the light-harvesting antenna

The overall energy transfer time through the light-harvesting antenna of *Rb. sphaeroides* was measured by exciting the antenna pigment at highest energy, B800, and probing the arrival of the excitations in the lowest-energy pigment B896. The results of such a measurement is shown in Fig. 3. The analyzing wavelength, 910 nm in the red wing of the LH1 absorption band, monitors the rise of the B896 excited state free of contributions from absorbance changes due to the main LH1 antenna (B875). The kinetic curve of Fig. 3 shows

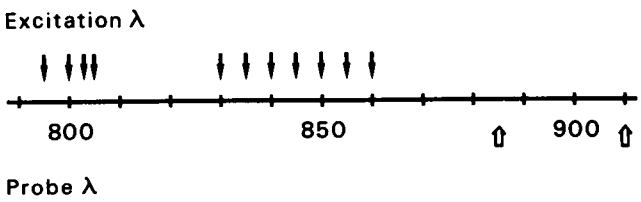

FIGURE 2 Summary of excitation and probing wavelengths used. Filled arrows indicate excitation wavelengths, while open arrows represent probe wavelengths.

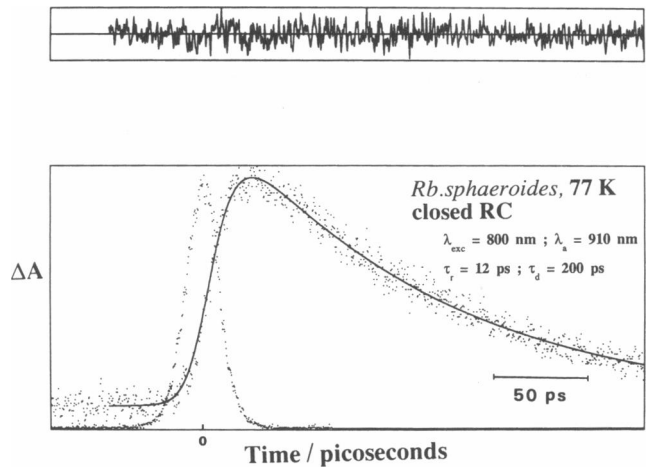

FIGURE 3 Rise-time kinetics of *Rb. sphaeroides* at 77K and 910 nm after excitation at 800 nm. Dots represent measured kinetics and the line is the best fit to data, yielding indicated rise and decay times. The upper panel represents residuals between fit and experimental values.

that the arrival of excitons to B896, after excitation of B800, is characterized by an exponential rise-time of 12 ± 1 ps. The same rise-time kinetics is observed for other excitation wavelengths within the B800 absorption band, suggesting no detectable heterogeneity of the B800 pigment, in agreement with earlier pump-probe experiments (22) and hole-burning studies (29) of the same band. The slow decay (~200 ps) of the B896 excited state reflects the quenching of the excitation energy by the closed reaction center (P^+) (8, 9).

LH2 to LH1 energy transfer

LH1 is known to be spectrally heterogeneous (9, 10, 18), consisting of a major B875 and a minor red-shifted B896 pigment. Energy transfer from LH2 to each of these pigments can be probed by the appropriate choice of excitation and probing wavelengths in the transient absorption experiment. The LH2 to B896 transfer time can be monitored by excitation within the B850 band (830–860 nm) and probing the rise-time of the bleaching of the B896 ground state at 910 nm, where there are absorbance changes only due to B896 (9). The rise and decay of the B875 excited state can, on the other hand, be monitored by exciting within the B850 band and probing the absorbance changes at 885 nm. At this wavelength only absorption changes due to B875 will be detected because this is the isosbestic wavelength of B896 (9).

The B896 kinetics is illustrated for two different excitation wavelengths, 830 and 850 nm in Fig. 4. The

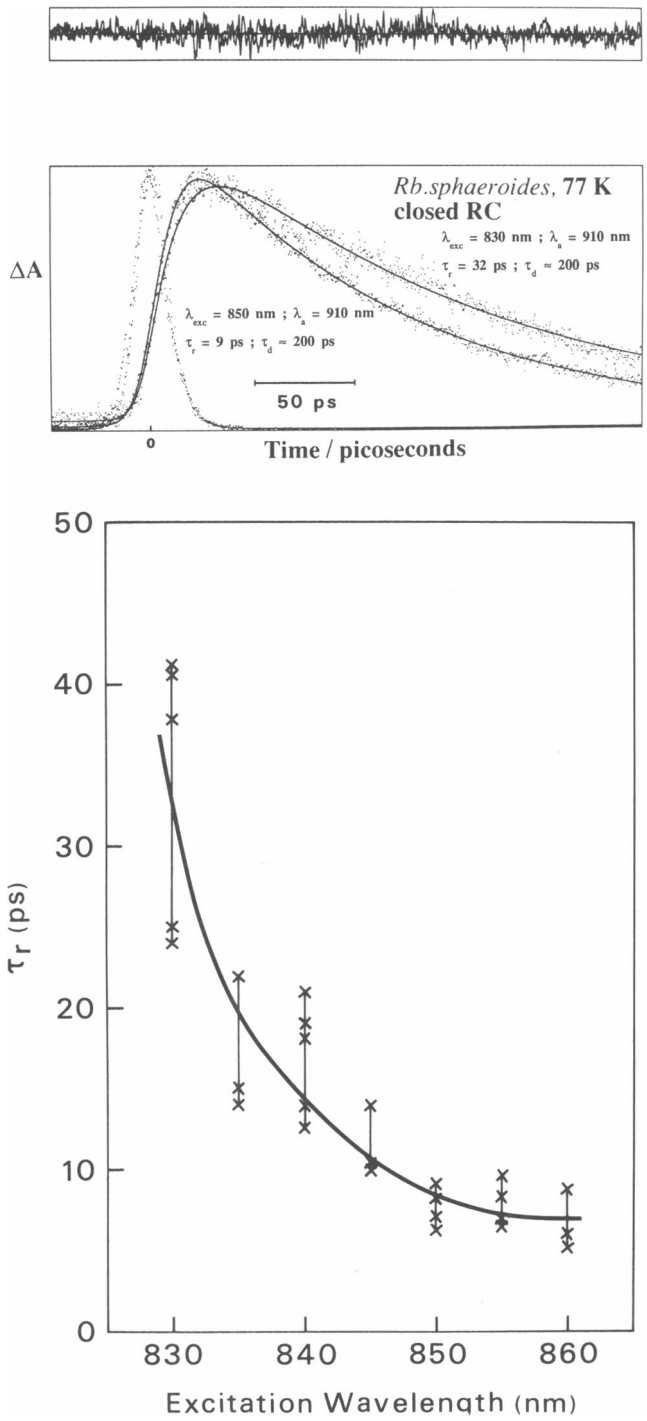

FIGURE 4 Rise-time kinetics of *Rb. sphaeroides* at 77°K and 910 nm after excitation at 830–860 nm. (*A*) (*Top*) Kinetics at two different excitation wavelengths. (*B*) Summary of wavelength dependence of the measured rise-time.

rise-time of the B896 bleaching signal is observed to be excitation wavelength dependent, while the ~200-ps decay reflecting the quenching by closed reaction centers (9) is wavelength independent. At λ_{exc} = 830 nm the rise-time is described by a 35 ± 5-ps time constant, while at 850 nm the corresponding rise-time is 9 ± 1 ps. When the rising part of the B896 signal is analyzed in terms of a single exponential, the resulting time-constant monotonically decreases from 35 ± 5 at 830 nm to 9 ± 1 at 860 nm. This is shown in Fig. 4 *B*.

We notice that the slow rise-time of the B896 excitons upon 830 nm excitation agrees very well with the decay time of B850 excitations measured at 830–840 nm in a single-wavelength experiment (9). At that time this result was interpreted in terms of a sequential energy transfer model, and was consistent with a relatively slow 35 ps energy transfer step from the main LH2 antenna (B850) to a minor red-shifted Bchl component (B870) of LH2, suggested to be the linking pigment between LH2 and LH1 (9, 11). The observed excitation wavelength dependence of the energy migration time from B850 to B896, suggests a more complex organization of the antenna than the previously suggested sequential scheme (9).

The kinetics of the B875 excited state was monitored at 885 nm for two different excitation wavelengths, 830 and 860 nm. The kinetic curve in Fig. 5 shows that the population of the B875 excited state rises with a time-constant of 2 ps or less, after excitation of B850 at 860 nm. The decay is dominated (>80% amplitude) by a 9-ps component, but it also carries a minor (<20% amplitude) 35-ps component. These two lifetimes agree

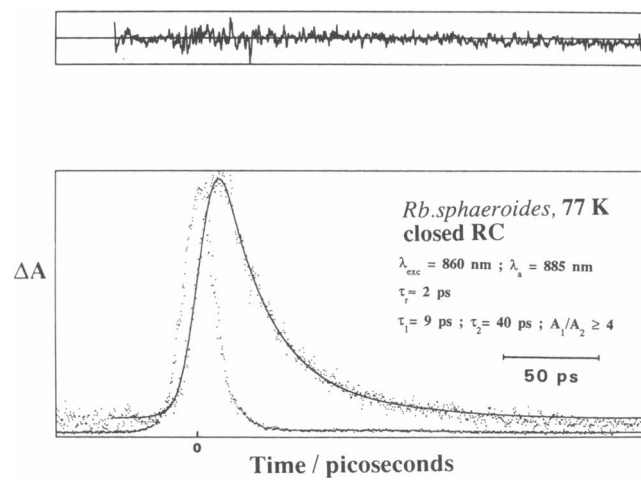

FIGURE 5 Measured kinetics of the B875 excited state of *Rb. sphaeroides* at 77K with closed reaction centers, excited at 860 nm and probed at 885 nm. The solid line is the best fit to the data.

with the two rise-times observed at 910 nm. Exciting the B875 kinetics at 830 nm and probing at 885 nm results in a qualitatively similar but quantitatively different time dependence (Fig. 6). The formation of the B875 excited state now appears to be characterized by a rise-time of ~5 ps, while the decay is described by a 35-ps component accounting for 50–80% of the amplitude and a 9-ps component of lower amplitude, 20–50%. Thus, upon changing the excitation wavelength from 860 to 830 nm, the rise-time of the B875 excited state lengthens from ≤2 to ~5 ps, while the amplitude of the 35-ps decay component increases. Another very distinct experimental observation is that the transient absorption signal excited at 830 nm has about a factor of five lower total intensity than the decay excited at 860 nm (corrected for differences in absorption at the two wavelengths). These features will be used in the following discussion (see Discussion part *e*) to construct a detailed kinetic model for the energy transfer through the LH antenna.

DISCUSSION

A model for the organization of the light-harvesting pigments in *Rb. sphaeroides*

In order to give a model of the energy transfer and pigment organization in the LH antenna of photosynthetic purple bacteria, we combine the present results with some previous picosecond results from this laboratory. The model is schematically illustrated in Fig. 7. In the following sections we will discuss each step in more detail.

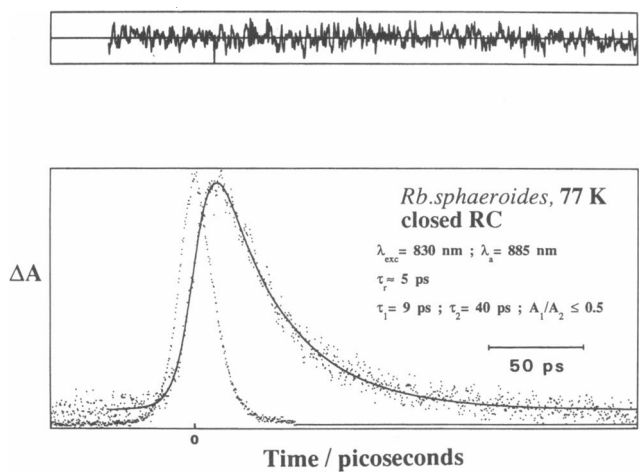

FIGURE 6 Measured kinetics of the B875 excited state in *Rb. sphaeroides* at 77K with closed reaction centers, excited at 830 nm and probed at 885 nm. The solid line is the best fit to the data.

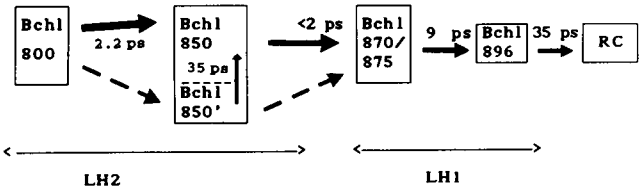

FIGURE 7 A kinetic model for the energy migration through the light-harvesting antenna of *Rb. sphaeroides* at 77K. The model describes the situation with photochemically active reaction centers.

(a) LH1 → RC energy transfer

Here we discuss the trapping of excitation energy by "open" photochemically active reaction centers. The slow ~200-ps decays observed in Fig. 3 and 4 reflect the decay of the B896 excited state due to quenching by closed reaction centers (P^+). The 35-ps trapping time at 77K was obtained by measuring the decay of B896 excitations after direct optical excitation of B896 in chromatophores with active primary charge separation (19, 20). The trapping time was found to be constant over the temperature range 177–77K, and we do not expect this rate to be much different at room temperature (at most a factor of two). The actual rate for the trapping step could not be directly measured at room temperature, because at this temperature it is not possible to measure the unidirectional transfer of energy from B896 to the special pair. Even if B896 is selectively excited by a picosecond pulse, the energy will be rapidly equilibrated over the LH1 antenna, and the resulting kinetics will largely reflect this process and the subsequent overall trapping of the equilibrated excitation density (8).

The measured trapping time of 35 ps at 77K was estimated to correspond to a B896-P870 distance of ~30 Å, which agrees quite well with what one can expect on the basis of size and shape of the reaction center (19). Earlier measurements (30) have indicated that the extent of detrapping is low. Assuming B896 and P870 to be isoenergetic at room temperature, results in a molecule-to-molecule detrapping rate of $(35\ \text{ps})^{-1}$. With four Bchl896 molecules per reaction center, the total detrapping rate is $(9\ \text{ps})^{-1}$, and the detrapping yield is 28%. Preliminary measurements of the detrapping in *Rhodospirillum rubrum* (25), performed by direct optical excitation of the 800 nm accessory-Bchl band of the RC and probing the appearance of antenna excitations, indicates that the detrapping yield is ~25% at room temperature and much less (<10%) at 77K. These results show that the trapping step B896 → P870 energy transfer is slow, much slower than other energy transfer steps within the antenna. The slow trapping combined with the fast primary charge separation results in a low detrapping

yield. The slow trapping rate necessitates a "focussing" pigment like B896 consisting of only a few antenna molecules, to make trapping sufficiently efficient. Thus, with the observed trapping time of 35 ps and a homogeneous LH1 antenna of ~30–50 Bchl molecules, the total trapping time at room temperature would be of the order of 350–500 ps, instead of the observed 90 ps (8). The longer trapping time at room temperature of *Rb. sphaeroides* (90 ps) and *R. rubrum* (60 ps) as compared with 77K is most likely also a result of the B875/B896 heterogeneity of the LH1 antenna, because at higher temperature the excitation energy is more extensively delocalized over the main LH1 antenna.

(b) Energy transfer within LH1; B875 → B896

Earlier steady-state and time-resolved work has shown that the LH1 antenna is heterogeneous from a spectral and energy transfer point of view, and consists of at least two spectral components, B875 and B896. From single-wavelength picosecond absorption measurements (9, 10), where B875 was selectively excited by a picosecond pulse, and the decay of B875 excitations was monitored, it was concluded that the energy transfer time from B875 to B896 is characterized by a 12–15-ps time constant. It seems reasonable to interpret the 9-ps rise-time of B896 excitations, observed in this work upon excitation in the wavelength range 850–860 nm, as due to the B875 → B896 transfer step. The slight discrepancy between the value of 9 ps obtained in this work for this step, as compared with the earlier value of 12–15 ps, is probably a result of the fact that we use a more elaborate deconvolution procedure to account for the finite width of the apparatus response function in our work.

(c) LH2 → LH1 energy transfer; B850/850′ → B896

The excitation wavelength dependence of the LH2 → B896 transfer time observed upon exciting within the B850 absorption band over the wavelength interval 830–860 nm and detecting the rise-time of the B896 bleaching at 910 nm (Fig. 4 *A* and *B*), strongly suggests that B850 is spectrally heterogeneous and that energy transfer from the spectrally different bacteriochlorophyll molecules to LH1 occurs with different rates. Various models could be envisaged for such a heterogeneity. The B850 absorption band could be inhomogeneously broadened and consist of many slightly different spectral forms of Bchl850, arising from a spectral distribution of B850 or from a distribution of B850–B875 distances, combined with a simultaneous spectral distribution. A similar model has been suggested by Freiberg et al. (13, 16) for the LH1 antenna of *R. rubrum*. However, these data hardly allow a more sophisticated analysis than that of describing the B850 as composed of two subsets with differing energy transfer rates to the LH1 antenna. This implies that the rise-time measured for the arrival of the excitons to B896 should be nonexponential (or biexponential in the case of two pigments). However, with the present time resolution of the measurement system and the relatively small difference in rise-times, the observed rise will be only marginally different from a single exponential one. Nevertheless, if we choose the two extreme rise-times of 35 and 9 ps to represent the transfer times from the two subsets of B850 to LH1, we can analyze the gradual change of the measured average rise-time in the excitation wavelength interval 830–860 nm (Fig. 4 *B*), as due to the overlapping absorption bands of the two spectrally different B850 components of which varying amounts are excited when the excitation wavelength is changed from 830 to 860 nm. Normalizing the sum of the two component spectra thus produced, to the steady-state absorption spectrum, results in the spectral decomposition shown in Fig. 8. The slowly rising (35 ps) kinetic component is characterized by a spectrum peaking at ~840 nm (B850′), and accounts for most of the absorption intensity in the 830–840-nm wavelength range. The fast 9-ps rise-time component is characterized by a spectrum with maximum at ~850 nm (B850) and accounts for more than 90% of the absorption intensity in the wavelength interval 850–860 nm.

(d) LH2 → LH1 energy transfer; B850/B850′ → B875

The excitation wavelength 860 nm selectively excites B850 with very little excitation of B875 (10, 18, 32). This means that the observed B896 excited state rise-time

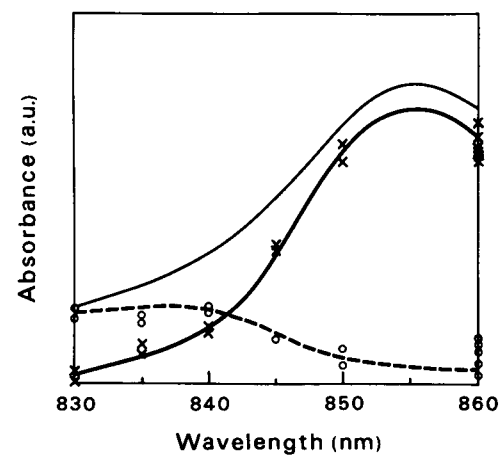

FIGURE 8 Excitation spectra of the two B850 forms. The x and o symbols represent the spectra of the 9- and 35-ps components, respectively. The sum of the two components is normalized to the steady-state absorption spectrum (*thin line*).

measured with 860 nm excitation and 910 nm probing (see Fig. 4 *A* for kinetic trace) includes the two energy transfer steps B850 → B875 → B896. Because the measured overall time constant for this process is within experimental error equal to the decay time of directly excited B875 (B875 → B896) (9, 10), the energy transfer from B850 to B875 must occur within, at most, a few picoseconds. A similar conclusion can be drawn from the kinetics of B875 excitations after excitation at 860 nm and probing at 885 nm (Fig. 5), displaying a <2-ps rise-time. Earlier single-wavelength picosecond absorption work (9) and steady-state spectroscopy (23, 31) of *Rb. sphaeroides* and an LH2-only mutant of *Rb. sphaeroides,* indicated the presence of a B870 pigment that was supposed to couple LH2 and LH1 in efficient energy transfer. This is indicated in the scheme of Fig. 7. We are presently investigating the properties and function of this component more closely.

The B875 kinetics observed after 830 nm (B850′) excitation provides information about the heterogeneity of B850 and the B850′ coupling to LH1. The kinetic model of Fig. 7, describing the energy migration in the light-harvesting antenna of *Rb. sphaeroides,* predicts deactivation of B850′ via a slow 35-ps step followed by a faster 9-ps decay of the B875 excitons. The consequence of this is that upon direct optical excitation of B850′ the concentration of B875 excited states will be low at all times, and 875 excited states will appear with a rise-time of 9 ps and decay with a time constant of 35 ps. This is seen from Fig. 9 and the expression below for the time dependence of the absorption signal monitoring the rise and decay of B875 excited states. For simplicity we have omitted B850 in this scheme, because it transfers very rapidly to B875.

$$\Delta A = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t)$$

$$A_1 = \Delta\epsilon_{875}(c^0_{875} - c^0_{850'}k_2(k_1 - k_2)^{-1}); \quad k_1 = (9\text{ ps})^{-1}$$

$$A_2 = \Delta\epsilon_{850'}c^0_{850'} + \Delta\epsilon_{875}c^0_{850'}k_2(k_1 - k_2)^{-1}; \quad k_2 = (35\text{ ps})^{-1}.$$

In these equations $\Delta\epsilon_{875}$ and $\Delta\epsilon_{850'}$ are the differences in extinction coefficients between ground and excited states of the respective pigments, and c^0_{875} and $c^0_{850'}$ are the initial excited-state concentrations of the two pigments. For excitation wavelengths in the interval 830–840 nm the initial concentration of excited Bchl875, c^0_{875}, can safely be set to zero. The negative sign of A_1, obtained with the given values of the rate parameters, implies that the rate constant belonging to this component k_1, will be observed as a rise-time. These expressions also show that the amplitude of the 35-ps component (A_2) should be ~¼th the amplitude of the observed decay with the opposite ratio of the rate constants, i.e., when $k_1/k_2 = ¼$, which for instance is the case for the B875 decay with 860 nm excitation. This model only considers direct optical excitation of B850′. Experimentally the situation is somewhat more complex because excitation within the B850/B850′ band generates a mixture of B850 and B850′ excited states in a ratio given by the absorption spectra of Fig. 8. The B875 kinetics will therefore be composed of contributions due to direct excitation of both B850 and B850′. The contribution to the B875 kinetics, due to directly excited B850, can be accounted for in a straight-forward manner because this process is expected to be described by a fast (<2 ps due to B850 → B875 transfer) rise-time and 9-ps decay due to B875 → B896 transfer.

FIGURE 9 Simplified kinetic model for B850′ → LH1 → RC energy transfer.

The measured kinetics of the B875 excited state upon 830 nm excitation is fully consistent with these predictions. Thus, the prediction of a 35- and a 9-ps decay time (the latter due to direct extinction of B850) is immediately verified, and the observed amplitude ratio of ~2:1 for the slow to fast decay component results in an ~1:10 absorbance ratio of B850/B850′ at 830 nm, when the ¼ diminishing factor of the amplitude of the B850′ → B875 process is taken into account. This is in good agreement with the excitation spectrum for these two pigments obtained from the rise-time measurements at 910 nm (Fig. 8). The B875 signal at 885 nm with 830 nm excitation is predicted to have a biexponential rise characterized by a ≤2- and a 9-ps component, due to directly excited B850 and B850′, respectively. With the present time resolution this is difficult to resolve and a weighted average of the two rise-times will only be observed, hence the observed ~5-ps rise-time. The ~⅕ intensity ratio of the B875 transient absorption signal excited at 830 (Fig. 6) and 860 nm (Fig. 5) is also in very good agreement with the predictions of this kinetic model. These results, demonstrating the presence of a LH2 heterogeneity, are in agreement with findings in time-resolved fluorescence measurements of a ~40-ps fluorescence rise-time component of the LH1 fluorescence detected at 940 nm (15, 16).

From these results it is difficult to determine the exact coupling of B850′ to the main LH2 and LH1 antennas. Whether the 35-ps liftime of B850′ represents a direct coupling to LH1 or a relatively inefficient coupling to the main B850 pigment of LH2 followed by efficient B850 → B875 transfer cannot be decided from these measurements. However, the fact that a similar ~40-ps lifetime

is observed in the NF57 LH2-only mutant (11), where there is no B850′ → LH1 energy transfer possible, suggests that the 35-ps lifetime to a large extent represents energy transfer from B850′ to B850. If this is indeed the case, the slow transfer may represent transfer between energetically less coupled pigment molecules and/or a longer transfer route (more energy transfer steps) through the LH2 antenna network. Energy transfer involving a B870 pigment, coupling the LH2 and LH1 antennas, is most likely extremely fast and would not be distinguished with the present time resolution. At present we have no explanation for the spectral inhomogeneity of B850, but we plan to investigate these and related effects in LH2-only mutants of *Rb. sphaeroides* and spectrally altered mutants. In this connection the possible involvement of exciton states should be mentioned. Similarly, heterogeneous energy transfer kinetics in allophycocyanin pigments of blue-green bacteria (33) and chlorosomes of green bacteria (34) have been interpreted as slow relaxation between exciton states. Theoretically, very little is known presently about the time-scale of interexciton state relaxation. However, crude estimates (35a, b) and experimental hole-burning results (36a,b) places this type of relaxation on the femtosecond to subpicosecond time-scale. Thus, we feel that exciton state dynamics is less likely to be the explanation to the heterogeneous nature of the B850 absorption band.

(e) Energy transfer from B800

From earlier time-resolved picosecond work (8, 9, 21, 22) the energy transfer time from B800 was estimated to 1–2 ps at 77K and somewhat faster at room temperature. Hole-burning experiments on an isolated LH2 pigment-protein complex at 1–30K (29) suggested a 2–3-ps lifetime of the B800 excitons. Very recent absorption measurements using 500 fs 800 nm pulses to excite B800 and probe the decay of the excited state, showed that energy is transferred from B800 to B850 with a 0.7 ± 0.2-ps time constant at room temperature and 2.2 ± 0.2 ps at 77K (Visscher et al. 1992, submitted for publication). These measurements only reveal the total deactivation rate of the B800 excited state, they do not provide information about the pathways of energy flow through the B800–850/850′ pigment complex. In particular, we would like to know whether energy absorbed by B800 is distributed over the B850/850′ pigments or if only B850, constituting the fast channel of the kinetic scheme in Fig. 7, is involved. To this end we compare the 12-ps rise-time of the B896 excited state after B800 excitation with simulated kinetics for the two different possibilities mentioned above; i.e., (*i*) the measured rise-time represents the effective rise-time for excitons migrating exclusively along the fast pathway of the kinetic scheme of Fig. 7, i.e., B800–850 $\xrightarrow{\tau_1}$ B875 $\xrightarrow{\tau_2}$ B896 $\xrightarrow{\tau_3}$ RC, with time constants for each individual step of $\tau_1 = 2.2$, $\tau_2 = 9$, and $\tau_e = 200$ ps. For the B800–850 → B875 transfer step we use for simplicity the recently measured vaue 2.2 ps for B800 → B850 transfer at 77K (Visscher et al. 1992. Submitted for publication), because we know that B850 → B875 is at least as fast (see Discussion part *d*). (*ii*) The 12-ps rise-time represents a weighted average of transfer along the fast and slow pathways of Fig. 7. The results of the simulations are depicted in Fig. 10 for two different contributions of transfer along the slow path, (*A*) 10%, and (*B*) 20% of the total amplitude. It is seen that transfer exclusively along the fast pathway generates kinetics (curve 2 of Fig. 10) practically indistinguishable (considering the noise of a typical measurement; see Fig. 3) from the measured 12-ps rise and 200-ps decay (curve 1), while the kinetic curve including a contribution from the slow path (curve 3) significantly deviates from the measured curve, already at 10% contribution from the slow 35-ps component. Thus, we conclude that more than 90% of the excitation energy

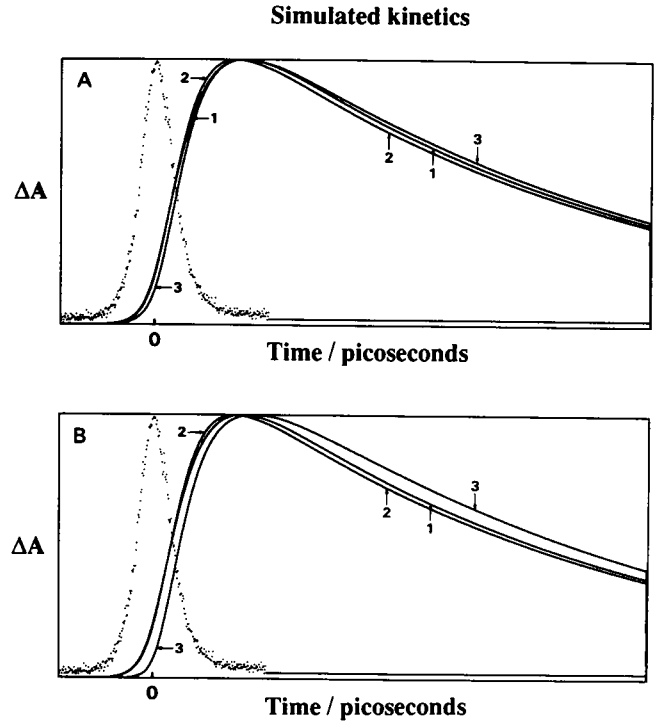

FIGURE 10 Simulated kinetics of the B896 excited state following B800 excitation. (*Curve 1*) Kinetics obtained from a fit to experimental data with $\tau_1 = 12$ ps ($A_1 = -1$) and $\tau_2 = 200$ ps ($A_2 = 1$); (*curve 2*) simulated curve for transfer along exclusively the fast route with $\tau_1 = 2.2$ ps ($A_1 = 0.25$), $\tau_2 = 9$ ps ($A_2 = -1.32$), and $\tau_3 = 200$ ps ($A_3 = 1.07$); (*curve 3*) same as curve 2 with a contribution added from a slow rise-time $\tau_4 = 35$ ps ($A_4 = 0.1(A_2 + A_4)$, Fig. 10 *A*; $A_4 = 0.2(A_2 + A_4)$, Fig. 10 *B*).

absorbed by B800 is transferred exclusively along the fast pathway.

SUMMARY

Using low intensity picosecond absorption spectroscopy with independently tunable excitation and probing infrared pulses, we have studied the pathways of energy transport through the light-harvesting antenna pigments of the photosynthetic purple bacterium *Rb. sphaeroides*. By measuring the rise-time of the B896 excited-state in the red wing of the LH1 absorption band (λ_p = 910 nm) after picosecond pulse excitation in the LH2 absorption bands (λ_{exc} = 800–860 nm), we conclude that LH2 is spectrally heterogeneous, consisting of a major B850 and a minor blue-sifted B850′ form. Energy transfer from B800 of LH2 is fast, 0.7 ps at room temperature and 2.2 ps at 77K, and transfer from B850 to LH1 is also fast, $\leq$2 ps. In contrast, energy transfer from the minor B850′ to LH1 is slow, 35 ps. This results in a fast and slow channel for energy transfer through the light-harvesting antenna. For light absorbed by the high energy pigment, B800, more than 90% of the energy follows the fast channel and reaches the lowest energy pigment of the antenna (B896) with a time constant of 12 ps.

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