Dynamics of energy transfer and trapping in the light-harvesting antenna of *Rhodopseudomonas viridis*

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ABSTRACT By low intensity picosecond absorption spectroscopy it is shown that the exciton lifetime in the light-harvesting antenna of *Rhodopseudomonas (Rps.) viridis* membranes with photochemically active reaction centers at room temperature is 60 ± 10 ps. This lifetime reflects the overall trapping rate of the excitation energy by the reaction center. With photochemically inactive reaction centers, in the presence of P^+, the exciton lifetime increases to 150 ± 15 ps. Prereducing the secondary electron acceptor Q_A does not prevent primary charge separation, but slows it down from 60 to 90 ± 10 ps. Picosecond kinetics measured at 77 K with inactive reaction centers indicates that the light-harvesting antenna is spectrally homogeneous. Picosecond absorption anisotropy measurements show that energy transfer between identical Bchl*b* molecules occurs on the subpicosecond time scale. Using these experimental results as input to a random-walk model, results in strict requirements for the antenna-RC coupling. The model analysis prescribes fast trapping (≈1 ps) and an ~0.5 escape probability from the reaction center, which requires a more tightly coupled RC and antenna, as compared with the Bchl*a*-containing bacteria *Rhodospirillum (R.) rubrum* and *Rhodobacter (Rb.) sphaeroides.*

INTRODUCTION

The primary photochemistry and structure of the photosynthetic reaction center of the photosynthetic purple bacterium *Rhodopseudomonas (Rps.) viridis* are extensively studied and thoroughly characterized (1–5). In sharp contrast to this stands the almost complete lack of knowledge of how the energy of light is captured by the light-harvesting antenna and transported to the reaction center. However, through the work of Trissl et al. (6) we have some knowledge about the time scale of overall trapping and charge separation. Using the picosecond photoinduced electric gradient technique, Trissl et al. (6, 7) estimated that energy trapping and charge separation in whole cells of *Rps. viridis* occurs within 40 ps, essentially independent of redox state of the secondary electron acceptor. Secondary electron transfer from bacteriopheophytin to the quinone was observed to be slower, 120 ps, and occurred only when the quinone was initially oxidized. Using the time-resolved fluorescence single photon counting technique, Bittersman et al. (8) measured the fluorescence decay times of Bchl*b* at 1025 nm after picosecond excitation at 600 or 830 nm of whole cells, membranes, and quantasomes of *Rps. viridis.* For dark adapted preparations these authors observe one major fluorescence lifetime of 40–80 ps (the longest lifetime was observed for whole cells), accounting for more than 90% of the total amplitude, which they attribute to the trapping and primary charge separation.

For other photosynthetic organisms, in particular Bchl*a*-containing purple bacteria (*Rhodobacter (Rb.) sphaeroides, Rhodospirillum (R.) rubrum, Rhodopseudomonas (Rps.) acidophila*), a detailed knowledge exists about the dynamics of energy transport and trapping within the light-harvesting antenna. For all purple bacteria investigated so far, trapping takes place within 60–70 ps (12, 13, 16, 17; Bergström et al., unpublished results). At low temperature (77 K), the LH1 antenna of all purple bacteria was found to contain a minor red shifted species that possibly couples the LH1 to the reaction center (14, 17, 18). The lack of knowledge about the energy transport dynamics and pigment organization of the *Rps. viridis* antenna is mainly a consequence of the lack of suitable picosecond laser sources to produce light-pulses in the wavelength range of Bchl*b* absorption, i.e., 960–1,050 nm.

In order to obtain information about the energy transport dynamics and organization of the *Rps. viridis* light-harvesting antenna, we have performed a picosecond absorption spectroscopy study of *Rps. viridis* membranes, using low intensity picosecond pulses tunable in the 900–1,020-nm wavelength interval. The results show that the excitation energy is trapped with a time constant of 60 ps by photochemically active reaction centers. With inactive reaction centers (P^+) the energy is still efficiently quenched, but this process occurs approximately three times slower. With the secondary electron acceptor Q_A prereduced, trapping and primary charge separation occurs with a somewhat modified rate, $(90\ ps)^{-1}$. Picosecond absorption measurements at 77 K indicate that the light-harvesting antenna is spectrally homogeneous.

0006-3495/92/03/694/10 $2.00

EXPERIMENTAL

In order to study energy transfer dynamics in a light-harvesting antenna consisting of several hundred pigment molecules coupled to each other by dipole-dipole interaction, several requirements of the picosecond pulse source and experimental conditions have to be met. In order to avoid singlet-singlet and singlet-triplet annihilation (9–12) of the excitons, which distorts the true dynamics of energy transfer, the excitation pulse energy must be sufficiently low so that less than one photon is absorbed per domain of coupled antenna molecules. The wavelength of the excitation and probing pulses has to be tunable over the long wavelength Q_y absorption band of the pigment molecules. For the Bchl*b*-containing *Rps. viridis,* this implies 960–1,050 nm. The measurements should be polarization sensitive. In this way kinetics can be measured which reflects either the population dynamics of the pigments (magic-angle polarization), or the time dependence of the absorption (fluorescence) anisotropy (parallel and perpendicular polarizations). In the latter type of measurement, information about energy transfer between identical but differently oriented pigment molecules can be obtained.

In this work, the dynamics of energy transfer was monitored with the picosecond absorption technique (13), using identical wavelength of excitation and probing pulses. This constitutes a limitation of the laser system at its present stage of development. Dual excitation and probe wavelength experiments in this region of the spectrum are in progress. Picosecond pulses were generated in a sync-pumped and cavity-dumped dye laser using the laser dye Styryl 13 which was purchased from Radiant Dyes Chemie (Wermelskirchen, Germany). With a three-plate birefringent filter as tuning element, ~10 ps wide pulses could be generated in the wavelength range 900–1,020 nm. At a pump power of 500 mW from the mode-locked CW Nd-YAG laser, ~10 mW of dye laser output at the peak of the dye emission band (950 nm) could be obtained at a repetition rate of 800 kHz. This was also the repetition rate used in all experiments, but the power to excite the sample was reduced to ~0.1 mW (corresponding to ~10^{12} photons cm^{-2} $pulse^{-1}$) to avoid distortions of the measured kinetics due to singlet-singlet and singlet-triplet annihilation. For the room temperature measurements the *Rps. viridis,* membranes were suspended in a 50 mM Tris buffer solution (pH = 8) and contained in a closed rotating cell (13) of 1 mm optical path length. At the rotation speed used, the illuminated volume of the sample at the waist of the two crossing laser beams was hit by approximately four laser pulses before completely renewed, and the revolution time of the cell was ~10 ms. With the pulse intensity used, ~1/1000th of the sample molecules were excited by each pulse. This effectively prevented accumulation of long lived intermediates. Kinetics was measured for three different redox states of the reaction center. Photochemically active ("open") reaction centers were achieved with 20 mM sodium ascorbate added to the sample, while no additions were used to generate photochemically inactive, "closed," reaction centers, with the primary electron donor of the reaction center in its oxidized form ($P960^+$). In the latter case, the continuous illumination from the high repetition rate laser pulse train (800 kHz) was sufficient to maintain the primary electron donor in its oxidized state. The secondary electron acceptor Q_A was prereduced by the addition of 50 mM sodium dithionite (i.e., with the reaction center in state PIQ_A^-). In order to ensure the identity of the reaction center redox state, care was taken to use deaerated solutions and perform measurements only on fresh preparations.

The measurements at 77 K were performed by preparing a buffered glycerol-water solution (glycerol:water = 3:1) of the membranes and the temperature was maintained with a liquid nitrogen cryostat. All experiments, including the room temperature ones, were performed with an optical density of ~0.3 at the peak of the absorption band. At 77 K, kinetics was measured with closed (P^+) reaction centers.

Measured kinetics is analyzed as a sum of exponentials, and lifetimes and amplitudes are obtained by using a nonlinear least-square fitting procedure. Each given lifetime represents the average of ~10 independent measurements on different samples, and the indicated errors are the observed scatter in lifetimes. Information about the early kinetics could in principle be obtained by deconvoluting the measured decays with the instrument response function. However, because the kinetics obtained in the present one-color experiments are distorted around $t = 0$ by the so-called coherence coupling artifact (19) (the spike seen in each kinetic trace at $t = 0$), no such attempts were made. The fitting starts at a delay-time where the coherence spike and pulse have died out. This results in a time resolution of ~5 ps. A major kinetic component of <5 ps time constant would still be observed in our measurements but the lifetime would be distorted due to the presence of the coherence spike. A very short lived component (<1 ps) of small amplitude could be masked by the spike and therefore escape detection.

RESULTS AND DISCUSSION

Room temperature kinetics with "open" reaction centers

Fig. 1 displays the picosecond time-resolved absorbance changes measured with magic-angle polarizations at 1,000 nm and room temperature in a membrane sample of *Rps. viridis* with open reaction centers. The kinetic curve is well described by a pulse-limited rise of an absorption signal, followed by a 60-ps decay to a low amplitude bleaching having a long lifetime, >10 ns.

The transient absorption is due to the excited state absorption of the Bchl*b* light-harvesting molecules, and the 60-ps decay time constant is assigned to the trapping of the excitation energy by the reaction center. The long lived bleaching signal after the initial 60-ps decay is due to the charge separated state P^+I^- that decays into $P^+Q_A^-$; the latter has a μs lifetime (15). The fact that we observe the bleaching of the P^+I^- state is evidence that the rate of energy trapping by the photochemically active reaction centers is measured. The single-wavelength technique used in these experiments excludes a more detailed study of the evolution of the P^+I^- state into the $P^+Q_A^-$ state at the maximum of the $P^+I^- - PI$ absorbance difference spectrum (970–980 nm), because the light-harvesting antenna of *Rps. viridis* has very low absorption in this region. Measurements with independently tunable excitation and probing wavelengths are currently in progress.

Kinetics measured with parallel ($I(t)_\parallel$) and perpendicular ($I(t)_\perp$) polarizations were used to construct the time dependent absorption anisotropy,

$$r(t) = (I_\parallel(t) - I(t)_\perp)(I(t)_\parallel + 2I(t)_\perp)^{-1}.$$

For *Rps. viridis* membranes at room temperature a low and time-independent (on our time scale) value of the

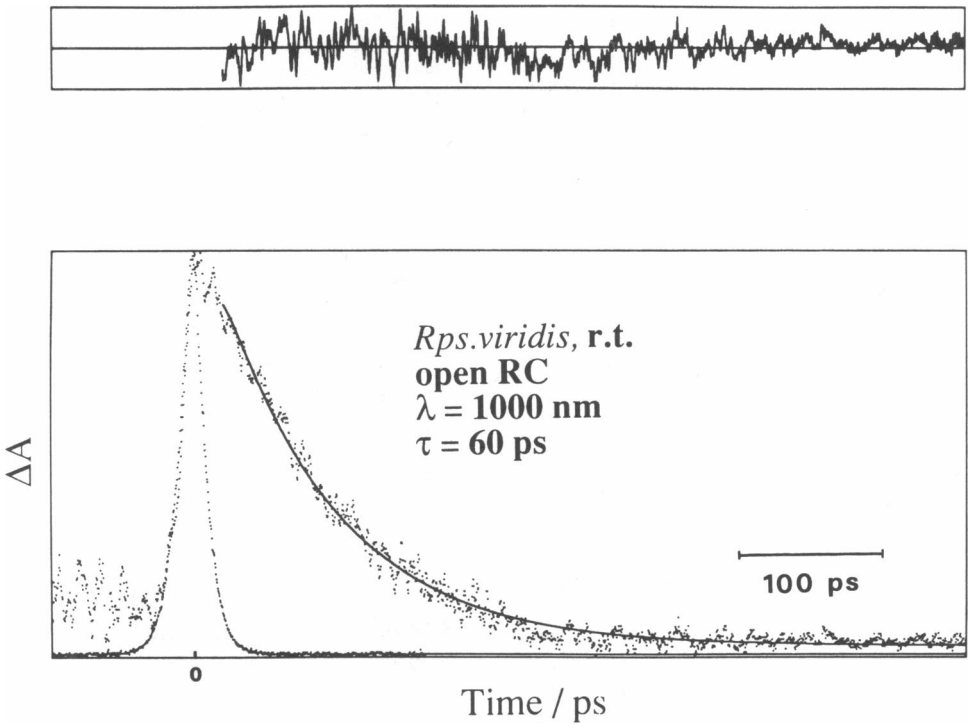

FIGURE 1 Picosecond absorption kinetics of *Rps. viridis* membranes with open RC at room temperature. An autocorrelation trace of the laser pulse is also shown in the figure. The upper panel in Figs. 1–4 represent a plot of the residuals between observed and fitted kinetics.

anisotropy was observed ($r(0) = r(\infty) \leq 0.09 \pm 0.02$) at all redox states of the reaction center. This is shown for the case of prereduced Q_A in Fig. 3 *B*. This suggests that there is a fast (subpicosecond) energy transfer process among differently oriented Bchl*b* light-harvesting pigment molecules preceding the trapping. Measurements of fluorescence polarization (25) led to a similar conclusion.

The results of the kinetic measurements illustrated in Figs. 1–4 were all obtained at an excitation density of $\sim 10^{12}$ photons cm^{-2} $pulse^{-1}$. This is already about a factor of 10–100 lower than used in most other similar measurements. In order to examine the possible influence of singlet–singlet and singlet–triplet annihilation, the kinetics was measured with different excitation pulse intensities. A variation of the excitation intensity by a factor of 10 (approximately threefold decrease and approximately fourfold increase of intensity from that used in Figs. 1–4) had no detectable effect, within the experimental accuracy, on the observed kinetics. This shows that the measured dynamics is not influenced by the mentioned processes.

The 60-ps lifetime observed in the present work for the energy trapping process in *Rps. viridis* is in reasonable agreement both with the time constant (40 ps) estimated by Trissl et al. (6, 7) from electric light-gradient measurements, and the value reported by Bittersman et al. for membranes and quantasomes (72 and 38 ps, respectively) (8). The trapping time at room temperature in several Bchl*a*-containing species (*R. rubrum* [13, 16], *Rb. sphaeroides* [13, 17, 18], *Rps. acidophila* [Bergström et al., unpublished results], and *Rps. capsulata* [Bergström et al., unpublished results]) has been found to range between 60 and 70 ps in all cases. The similarity of overall trapping time is quite remarkable, considering the apparent uphill energy transfer from antenna to the special pair in *Rps. viridis*. Below we will discuss possible explanations to this apparent similarity of exciton lifetimes in the different species.

Room temperature kinetics with "closed" reaction centers (P^+)

At room temperature, closed reaction center conditions were established by the quasi-continuous illumination of the high repetition rate excitation pulse train, when no artificial electron donors were added to the sample. The results of an isotropic kinetic measurement at 1,000 nm is shown in Fig. 2. The transient absorption due to Bchl*b* is seen to rise with a pulse-limited rate and the decay is single exponential with a time constant of ~150 ps. Decays characterized by the same time constant were

observed in the whole investigated wavelength interval, 990–1,020 nm. These results show that, similar to the situation in Bchl*a*-containing purple bacteria, P^+ is an efficient quencher of excitation energy and the process occurs with about the same rate in both types of bacteria.

Room temperature kinetics with prereduced secondary electron acceptor (PIQ_A^-)

When the secondary quinone electron acceptor of the reaction center is chemically prereduced (using sodium dithionite), the photochemistry of the reaction center is blocked. However, earlier work on membrane preparations as well as on isolated reaction centers of *R. rubrum*, *Rb. sphaeroides*, and *Rps. viridis* (20–24) have shown that the state P^+I^- is still formed when Q_A is reduced. Similar observations have been made for green plants, and there the charge separation appears to slow down significantly as a consequence of the reduction of Q_A. The absorption kinetics of *Rps. viridis*, measured under strongly reducing conditions (Q_A^-) (Fig. 3), shows that the antenna excited state decays with a time constant of ~90 ps. The long lived low intensity bleaching after the initial 90-ps decay demonstrates the formation of P^+I^-. The somewhat longer decay time, as compared with open reaction centers, is most likely a result of slower rates of primary and secondary charge separation in the reaction center of *Rps. viridis* (see model analysis of the trapping process, below). This change of charge separation rate could in principle also alter the yield of the charge-separated radical pair P^+I^-. However, a relatively small change is expected, which will be difficult to observe in the present measurements, due to the low intensity of the P^+I^- bleaching. Bittersman et al. (8) also found a longer antenna lifetime (160 ps) upon Q_A reduction, but Trissl et al. (6, 7) conclude that primary charge separation occurs with a rate that is essentially independent of RC redox state.

77 K kinetics with closed reaction centers (P^+)

The light harvesting antenna of Bchl*a*-containing purple bacteria has been shown to be spectrally inhomogeneous. Thus, the LH1 core antenna of *R. rubrum* and *Rb. sphaeroides* contains a minor spectral component B896 in addition to the main B880/875 form. This minor component was proposed to focus the excitation energy in the vicinity of the reaction center and to provide a special entry to the reaction center special pair (23, 24). In order to investigate the possible spectral heterogeneity in *Rps. viridis*, we measured the picosecond absorp-

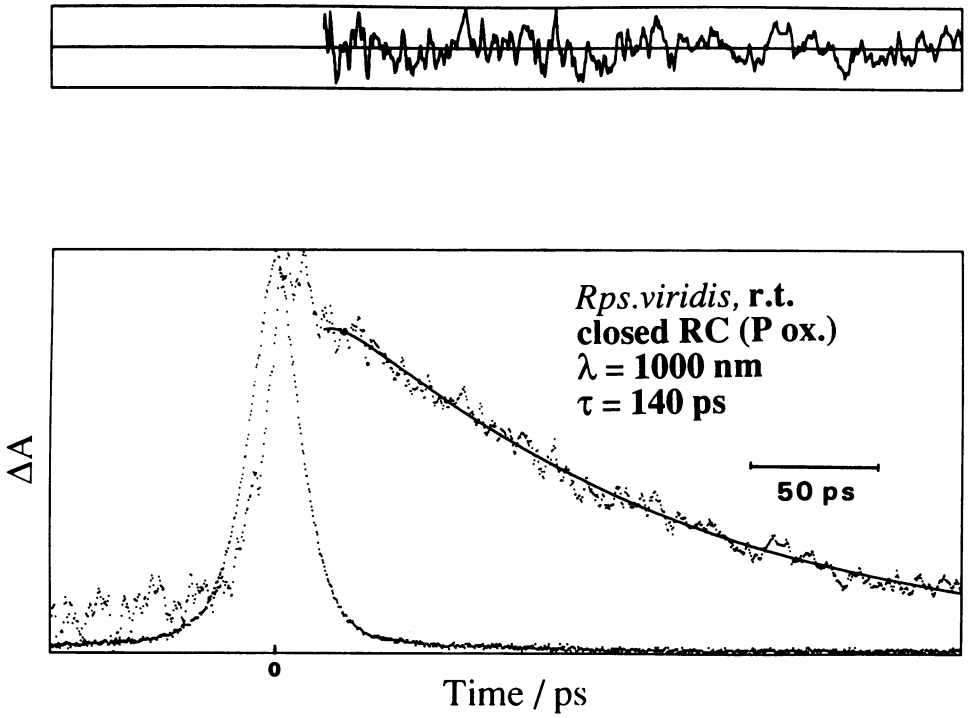

FIGURE 2 Picosecond absorption kinetics of *Rps. viridis* membranes with closed RC at room temperature.

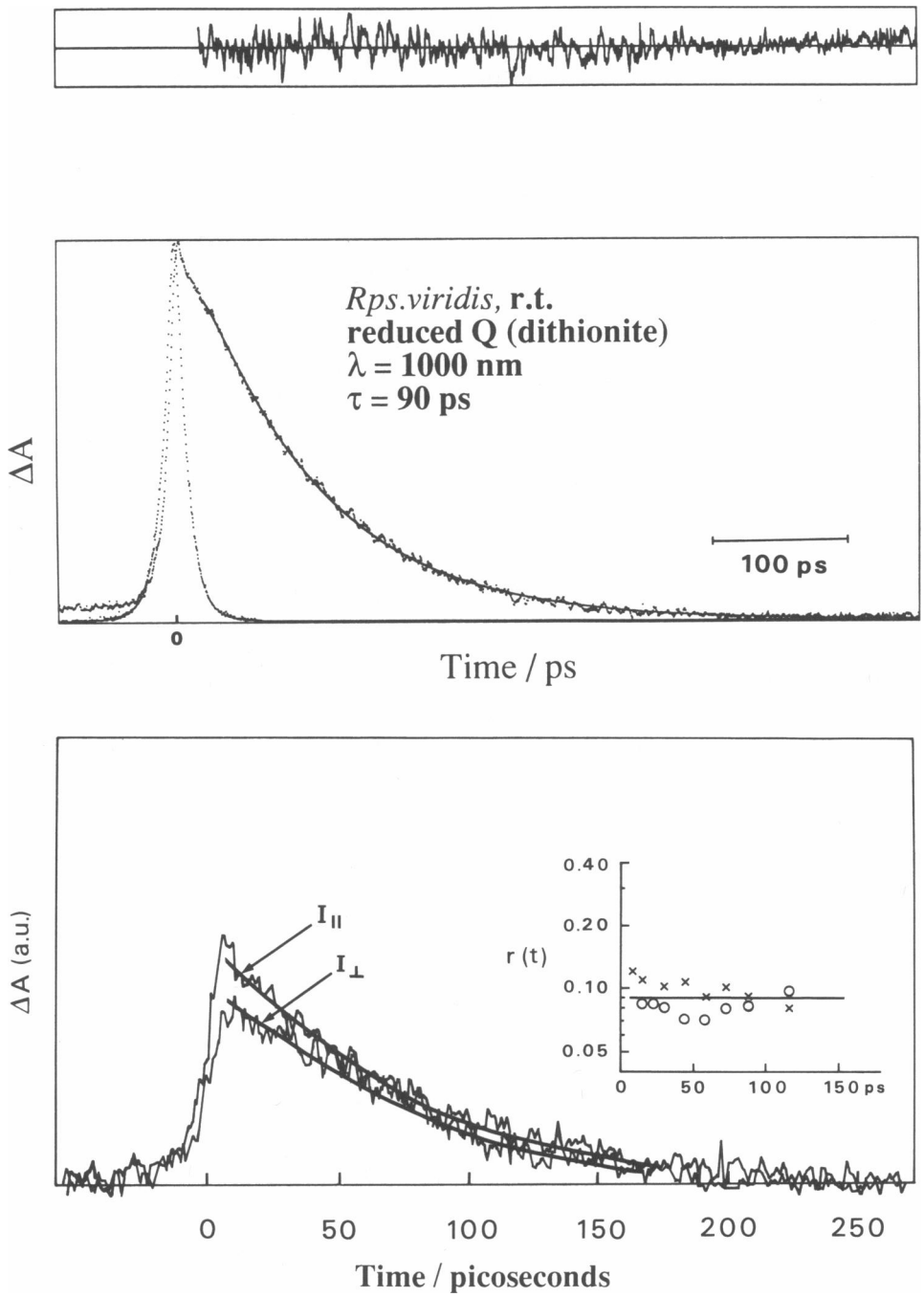

FIGURE 3 Picosecond absorption kinetics of *Rps. viridis* membranes with prereduced Q_A; (*A*) Isotropic kinetics. (*B*) Time-resolved absorption anisotropy, showing a measurement with parallel $I_\parallel$ and perpendicular $I_\perp$ polarizations. The inset shows the resulting anisotropy from two different measurements (o, x) and a line fitted to their average (——).

tion kinetics of membranes at 77 K in the wavelength range 990–1,020 nm. Previous experience from similar studies on *R. rubrum* (13) shows that a spectral heterogeneity of the type discussed here is best observed in the blue part of the absorption band. Fig. 4 shows the result of a picosecond absorption measurement of *Rps. viridis* membranes at 77 K with closed reaction centers. The kinetics is well described by a single exponential decay ($\tau = 150 \pm 15$ ps), very similar to that observed at room temperature, Fig. 2. Kinetics measured in the wave-

length interval 990–1,020 nm were also single exponential and yielded the same time constant, within experimental error. This suggests that the light harvesting antenna of *Rps. viridis* is spectrally homogeneous from an energy transfer point of view. From the low and wavelength-independent fluorescence polarization of *Rps. viridis* quantasomes, Breton et al. (25) arrived at a similar conclusion. This behavior is clearly distinct from the situation prevailing for *R. rubrum* and *Rb. sphaeroides,* where the energy transfer dynamics of the LH1 antenna strongly suggests the existence of, at least, two spectral forms.

Trapping dynamics in *Rhodopseudomonas viridis*

We have seen that the light-harvesting antenna of *Rps. viridis* appears to be homogeneous, consisting of Bchl*b* molecules with identical excited singlet-state energies, while *R. rubrum* and *Rb. sphaeroides* have a heterogeneous antenna. These differences in pigment composition will probably cause differences in details of the trapping dynamics of the two types of bacteria, despite the similarities suggested by the close correspondence of the measured trapping times at room temperature with open reaction centers.

In order to obtain more details about the trapping dynamics, we can analyze the measured trapping time using a model of the light-harvesting reaction center pigment system. Since the experimental results suggest a homogeneous light harvesting antenna in *Rps. viridis,* it appears reasonable to describe the antenna as an ordered network of pigment-protein complexes through which the excitation energy migrates by a diffusive random walk of sequential Förster energy transfer steps over nearest-neighboring antenna molecules. The coupling between the antenna and the reaction center can be thought of in different ways. One possibility is that the antenna and primary donor (P) of the reaction center are iso-energetic and that there is equal probability for the exciton to be on an antenna molecule and P. Provided there is very fast exciton jumping between neighboring molecules (as suggested by the anisotropy measurements), the experimentally observed lifetime τ_{ex} is given by $\tau_{ex} = N\,\tau_{ct}$, where N is the number of pigment molecules and τ_{ct} (= 2.8 ps [21]) is the room temperature time constant of primary charge separation in isolated reaction centers. With $N = 25$, $\tau_{ex} = 70$ ps, which is quite close to the observed trapping time constant.

In this simple model we assumed trapping and detrapping to occur with the same rate. A more realistic estimate of the exciton lifetime should be obtained by using the actual excited-state energies of P and the antenna as determined from absorption and fluorescence spectra. Thus, it is desirable to use a more general model of the trapping dynamics, allowing for a variable trapping efficiency and different trapping and detrapping rates. The model developed by Pearlstein (27, 28) provides such a description. This model is schematically represented in Fig. 5 with indicated rate constants for the various energy transfer steps. It should be emphasized that the light-harvesting antenna in this model is assumed to be a homogeneous antenna, consisting of identical pigment molecules.

In this model the exciton lifetime in the antenna, τ_{ex}, consists of two parts: the first passage time τ_{fpt}, which

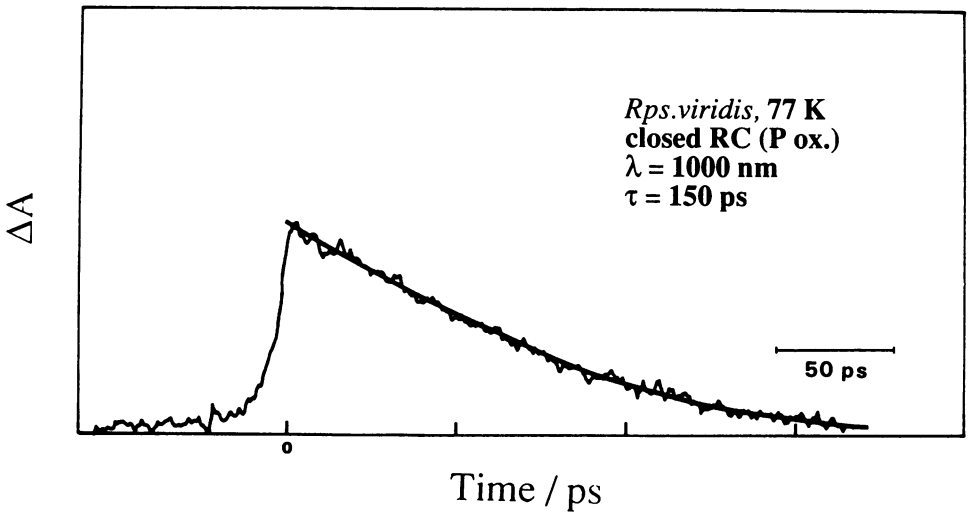

FIGURE 4 Picosecond absorption kinetics of *Rps. viridis* membranes with closed RC at 77 K.

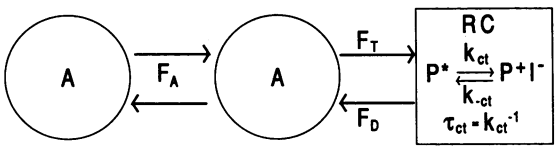

FIGURE 5 The Pearlstein model of the antenna-RC pigment system of *Rps. viridis*.

describes the random walk of the exciton from the site of photon absorption to the reaction center, and the revisiting time, which accounts for escape from the reaction center and subsequent revisits. The antenna exciton lifetime is equal to the experimentally available excited state lifetime, and is given by the following expression (28),

$$\tau_{ex} = \tau_{fpt}(1 - \rho) + \tau_{ct}[1 + (N - 1)I_D/I_T], \quad (1)$$

where ρ is the probability for direct optical excitation of the reaction center, N is the number of lattice sites and I_D and I_T are the Förster overlap integrals for the P* → antenna (detrapping) and antenna* → P (trapping) energy transfer processes, respectively. The first passage time τ_{fpt} is defined by

$$\tau_{fpt} = [(qF_T)^{-1} + (\alpha - q^{-1})F_A^{-1}]N, \quad (2)$$

where $\alpha = C_1(\ln N) + C_2$ for a two-dimensional lattice, q is the lattice coordination number, C_1 and C_2 are constants of order 0.1–0.4 (depending on lattice type and dimensionality [27]), and F_T and F_A are defined in Fig. 5. Here it should be noticed that the same coordination number q is used for an antenna lattice site and the reaction center. In view of the large size, elliptical symmetry (4, 5), and possible dimeric nature (26) of the reaction center this assumption may not be correct. In reality, the coordination around the reaction center may be much lower than around an antenna polypeptide. In a very recent extension of his model, Pearlstein considered a monocoordinate reaction center (29). The refined model is also extended in the description of the processes within the reaction center, and includes the secondary charge separation step. The predictions of this model are qualitatively similar to those of the isocoordinate model. In what follows we will analyze our results for *Rps. viridis* in terms of the original model, and whenever substantial differences occur between the two models, both will be discussed.

The exciton lifetime is said to be trap limited if there are multiple revisits to the antenna and the exciton lifetime dominated by the second term of Eq. 1. In the opposite case, when the first passage time is dominating, the exciton dynamics is said to be diffusion limited. From the expression for the first passage time, it is seen that the model accounts for different step times for homotransfer between antenna molecules $(qF_A)^{-1}$ and transfer between antenna molecules and the special pair of the reaction center $(qF_T)^{-1}$. This makes it possible to relate experimental observations of a real light-harvesting antenna system to predictions of the model.

By comparing the antenna exciton lifetime predicted by the model with the experimentally observed value, we can obtain some characteristics of the trapping process. Assuming an antenna homo-transfer rate of $> 10^{12}$ s^{-1}, consistent with the absorption anisotropy measurements, Eq. 2 can be reduced to (justified by the value of F_T obtained below)

$$\tau_{fpt} \approx (qF_T)^{-1}N. \quad (3)$$

For the revisting time we get,

$$\tau_{rev} \geq \tau_{ct}(N - 1)I_D/I_T = 2.8(N - 1)10^{-12}I_D/I_T. \quad (4)$$

We now estimate the magnitude of these two contributions to the exciton lifetime. From Eq. 3 it is seen that for a given antenna size the first passage time depends on the trapping rate (qF_T). We do not know the value of F_T for *Rps. viridis*, but as a first estimate we use the value estimated for the Bchl*a*-containing purple bacteria *R. rubrum* and *Rb. sphaeroides* (23, 24). For these species, trapping was shown to occur with a time constant of 35 ps in the temperature range 100–177 K, and estimated to be at the most a factor of two faster at room temperature. A similar value (40 ps) was estimated by Trissl et al. (7) for *Rps. viridis* from a calculation of the Förster overlap integral of antenna fluorescence and P absorption using a distance of 20 Å. The sixfold symmetry of the pigment-protein complexes surrounding the reaction center, observed in EM micrographs of *Rps. viridis* quantasomes (30), may indicate that there is a sixfold coordination around the reaction center, i.e., $q = 6$ in Eq. 3. Using the trapping rate $F_T = (17.5 \text{ ps})^{-1}$ estimated for the Bchl*a*-containing bacteria, this results in a first passage time of $\tau_{fpt} = 73$ ps. It is seen that with the chosen parameters, already the first passage time exceeds the measured antenna exciton lifetime. In the monocoordinate model this becomes even more pronounced, because q in Eq. 3 is reduced by a factor of six and thus the first passage time increases by the same factor with other parameters constant. Regardless of which coordination model is chosen, it appears clear that the trapping rate F_T has to be much faster than the assumed value. The first passage time could be reduced by increasing the trapping rate F_T, increasing the coordination number q, or by decreasing the effective antenna size N (see Eq. 3). Of these possibilities the first appears to be the most likely alternative, because q rather is less

than six as mentioned above, and our experimental results strongly suggest that the antenna is homogeneous. The trapping rate F_T could be increased by decreasing the distance between P and the antenna molecules transferring energy to P. A decrease of this distance by as little as 17% from the value used above (to ~17 Å) would reduce the trapping time F_T^{-1} and thus the first passage time, with a factor of three. Thus, with $F_T^{-1} = 6$ ps, $q = 6$, and $N = 25$, we get $\tau_{fpt} = 24$ ps. A distance of ~17 Å implies that the antenna and reaction center are very tightly coupled. In the monocoordinate model, a value of $F_T^{-1} = 1$ ps is required to obtain the same trapping rate (qF_T), necessitating an even tighter RC-antenna coupling.

In order to estimate the revisiting time τ_{rev} a value is needed of the Förster overlap ratio I_D/I_T in Eq. 4. Calculating this ratio on the basis of P absorption and fluorescence spectra measured on isolated reaction centers (31), and antenna spectra measured on membrane preparations (32), yields an overlap ratio $I_D/I_T \approx 5$. This results in a revisiting time $\tau_{rev} \approx 350$ ps and a total exciton lifetime $\tau_{ex} \approx 430$ ps, which is about a factor of 7 longer than the measured value. However, from photobleaching spectra of *Rps. viridis* (33, 34), measured on chromatophores, yielding the P^+IQ^--PIQ difference absorption spectrum, it appears that the absorption spectrum of P for an intact antenna-RC system is red-shifted by ~20 nm, relative to the spectrum in isolated reaction centers. Taking this shift into account and assuming a similar shift for the P fluorescence spectrum, an overlap ratio $I_D/I_T = 1.2$ is obtained. This latter value of the overlap ratio leads to a revisiting time $\tau_{rev} = 81$ ps. Together with the first passage time calculated above for the isocoordinate model, this gives a total exciton lifetime of $\tau_{ex} = 105$ ps, which is less than a factor of 2 longer than the experimental value. A similar overlap ratio ($I_D/I_T = 1.1$) was also obtained by using the fact that at 6 K there is very little (<10%) back energy transfer from P to the antenna (34). The observed low detrapping yield at 6 K is consistent with a detrapping rate $\leq (40 \text{ ps})^{-1}$ for the isocoordinate model. Together with the observed trapping time 1.3 ns at 6 K this results in an energy difference between P and antenna of =0.17 kJ mol^{-1}. Provided this difference is the same at room temperature, $I_D/I_T = 1.1$.

These results show that the exact value of the calculated exciton lifetime is very sensitive to the spectra used in the calculation, and that these spectra are sensitive to interactions between the components of the whole pigment-protein system. The remaining discrepancy between calculated and observed exciton lifetimes could possibly be decreased further with more detailed knowledge about the absorption and emission spectra of P with the reaction center in its native environment. In order to have full agreement between the observed exciton lifetime and that calculated with the isocoordinate model, the Förster overlap ratio would have to be $I_D/I_T \leq 0.5$. This would result in a detrapping yield at room temperature of ~0.5, in agreement with early estimates based on measurements of antenna fluorescence after excitation of the 830 nm RC absorption band (35). In the monocoordinate model (29), Pearlstein suggests that the antenna should be described as dimers of strongly interacting Bchl molecules, and that each lattice point, thus, is occupied by a dimer and $N = 12$. This description of the antenna implicitly assumes that an exciton remains delocalized over a dimer during a period of time at least corresponding to the hopping time between lattice points, i.e., on the order of a few hundred femtoseconds to a picosecond. If this assumption is correct, the calculated exciton lifetime, with all other parameters as given above, would be ~55 ps, in excellent agreement with the observed lifetime.

The distance ~17 Å, required for a reasonable agreement between observed and calculated exciton lifetimes, clearly implies very tight coupling between antenna and reaction center. For comparison, the corresponding distance for *R. rubrum* and *Rb. sphaeroides* was estimated to be ~30 Å (23, 24), consistent with the slow trapping observed in these bacteria. A distance on the order of 17 Å between two Bchl chromophores is typical for intracomplex distances (9, 13, Visscher, K. J., M. Oskarsson, R. van Grondelle, and V. Sundström. Ultrafast energy transfer from B800 to B850 in the LH2 antenna of photosynthetic purple bacteria. Submitted for publication.), and could result in substantial chromophore–chromophore interaction. Thus, it appears plausible, that the observed spectral blue-shift of P upon reaction center isolation is a consequence of breaking the antenna-P interactions. In the extreme limit of this interaction model, the antenna molecules and the special pair form a strongly exciton-coupled aggregate with the excitation energy delocalized over the participating chromophores. The exciton density on P would then depend on how the excited-state wave functions of the component molecules are combined into the exciton states.

Trapping with Q_A prereduced

We are now in a position where we can rationalize the observed increase of the trapping time upon prereduction of the secondary electron acceptor Q_A. Eq. 4 suggests that the increased trapping time may be a consequence of a decreased rate of primary charge separation (τ_{ct}^{-1}) upon reduction of Q_A. A reduction of this rate by approximately a factor of two would account for the observed change of the trapping time constant.

This agrees well with picosecond measurements on isolated *Rps. viridis* reaction centers, which indicate that the rate of primary charge separation slows down from $(2.8\ ps)^{-1}$ (reference 21) to $(5.6\ ps)^{-1}$ (reference 36) upon prereduction of Q_A. By including the secondary charge separation step in the monocoordinate model, Pearlstein showed that the antenna exciton lifetime depends on both the rate of primary and secondary charge transfer (29). With Q_A prereduced, there is no secondary charge transfer and the reoxidation of I^- becomes very slow, which results in an increased exciton lifetime. Thus, the observed increase from 60 to 90 ps of the exciton lifetime upon reduction of Q_A probably is a combined effect of decreased rates of primary and secondary charge separation.

CONCLUSIONS

The overall trapping time of excitation energy in the light-harvesting antenna of *Rps. viridis* was measured with low intensity tunable infrared picosecond pulses, and compared with the prediction of a random-walk model describing the reaction center as an isocoordinate trap or a monocoordinate trap. In order to obtain agreement between measured and calculated exciton lifetimes, it is necessary to use absorption and fluorescence spectra of the special pair (P), reflecting the actual interaction between P and its surroundings in the intact system; using the blue-shifted spectra of isolated reaction centers results in a theoretical exciton lifetime that is much too long. It is also necessary to assume a very tight coupling (~ 17 Å) between the antenna and the special pair of the reaction center. These results suggest that consideration of interactions between P and its surroundings (pigment-pigment and/or pigment-protein), causing spectral red-shifts of P, is essential in understanding the observed efficient trapping in *Rps. viridis*.

We thank professor R. M. Pearlstein for communicating his manuscript to us before its publication.

The Swedish Natural Science Research Council, the Swedish Institute, and the Dutch Foundation of Biophysics are gratefully acknowledged for financial support.

Reserved for publication 23 April 1991 and in final form 15 October 1991.

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