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TRAPPING, LOSS AND ANNIHILATION OF EXCITATIONS IN A PHOTOSYNTHETIC SYSTEM

II. EXPERIMENTS WITH THE PURPLE BACTERIA *RHODOSPIRILLUM RUBRUM* AND *RHODOPSEUDOMONAS CAPSULATA*

J.G.C. BAKKER [a], R. VAN GRONDELLE [a,b] and W.T.F. DEN HOLLANDER [c]

[a] *Vakgroep Biofysica, Huygens Laboratorium, Postbus 9504, 2300 RA Leiden,* [b] *Vakgroep Biofysica, Natuurkundig Laboratorium der Vrije Universiteit, de Boelelaan 1081, 1081 HV Amsterdam, and* [c] *Instituut-Lorentz voor Theoretische Natuurkunde, Nieuwsteeg 18, 2311 SB Leiden (The Netherlands)*

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In this paper a number of experiments with the purple bacteria *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata* is described in which the total fluorescence yield and/or the total fraction of reaction centers closed after a picosecond laser pulse were measured as a function of the pulse intensity. The conditions were such that the reaction centers were either all in the open or all in the closed state before the pulse arrived. These experiments are analysed using the theoretical formalism discussed in the preceding paper (Den Hollander, W.T.F., Bakker J.G.C., and Van Grondelle, R., Biochim. Biophys. Acta 725, 492–507). From the experimental results the number of connected photosynthetic units, λ, the rate of energy transfer between neighboring antenna molecules, k_h, and the rate of trapping by an open reaction center, k_t^o, can be estimated. For *R. rubrum* it is found that $\lambda = 14$–17, $k_h = (1$–$2) \cdot 10^{12}\ s^{-1}$ and $k_t^o = (4$–$6) \cdot 10^{11}\ s^{-1}$, for *Rps. capsulata* $\lambda \approx 30$, $k_h \approx 4 \cdot 10^{11}\ s^{-1}$ and $k_t^o \approx 3 \cdot 10^{11}\ s^{-1}$. The findings are discussed in terms of current models for the structure of the antenna and the kinetic properties of the decay processes occurring in these purple bacteria.

1. Introduction

As was outlined in the preceding paper [1], the study of the total (bacteriochlorophyll) fluorescence yield and the total fraction of reaction centers closed in a photosynthetic system after a picosecond light pulse, as a function of the pulse intensity, admits of a determination of the number of connected photosynthetic units, λ, forming a so-called 'domain'. Further, with the use of a random-walk model, describing the transfer of the excitations in a domain, estimates can be obtained of the molecular rate constants associated with the energy transfer process.

In this paper we analyze and discuss a series of experiments, performed with the purple bacteria *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata,* to which we have applied the formalism described in Ref. 1. In the following we shall therefore limit the discussion to photosynthetic bacteria. We shall briefly summarize the main points.

Using the bacteriochlorophyll fluorescence yield

Abbreviations: B800, B850 and B880, bacteriochlorophyll species absorbing at 800, 850 and 880 nm, respectively; BChl, bacteriochlorophyll; *R. rubrum, Rhodospirillum rubrum; Rps. capsulata, Rhodopseudomonas capsulata*; P, reaction center bacteriochlorophyll dimer; Bph, reaction center bacteriopheophytin; Q_1, reaction center primary quinone acceptor; log, natural logarithm.

as a monitor for the loss in the energy transfer process, Vredenberg and Duysens [2] concluded that in the purple bacterium *R. rubrum* a large number of photosynthetic units appear to form a 'lake' or 'matrix', in which the reaction centers share a common pool of antenna pigment molecules. Their conclusion was based on the experimental observation that the (low intensity) total antenna fluorescence yield, ϕ_f, as a function of the macroscopic fraction of closed reaction centers in the system, $\bar{x}$, is given by the hyperbolic relation:

$$\phi_f = \phi_f^o/(1 - p\bar{x}) \tag{1}$$

where ϕ_f^o is the total fluorescence yield observed with all the reaction centers in the open state and p is a parameter that reflects the difference in trapping strength between the open and the closed state (Refs. 2 and 3; Eqns. 1 and 17b of Ref. 1). Later Duysens [3] argued that Eqn. 1 can be properly explained only if the rate constant for energy transfer between a given pair of neighboring antenna molecules, k_h, exceeds by far the trapping rate constants of the open and the closed reaction center, k_t^o and k_t^c, respectively. Experiments by Rijgersberg et al. [4] have indicated that such is indeed the case, at least at room temperature. However, it should be stressed that $k_h \gg k_t^o, k_t^c$ is not a necessary condition for Eqn. 1 to hold and that deviations from Eqn. 1 are small even for relatively low rates of energy transfer [1]. Thus, even though only in the limit $\lambda \to \infty$ and $k_h \to \infty$ Eqn. 1 is exact [3,5], it is fairly accurate for a wide range of λ and k_h values [1]. What is interesting to note is that the two parameters in Eqn. 1 are both calculated from a single photosynthetic unit (Eqns. 6 and 17 in Ref. 1).

The relation (1) has been observed for several bacterial photosynthetic systems, under a wide range of conditions [6–10]. The quenching of the BChl fluorescence yield by carotenoid triplet states or quinones has, in most cases, also been explained in terms of the 'lake model' [8,11]. Deviations from Eqn. 1 were usually interpreted as to indicate a separation of the photosynthetic units [6,12,13].

The phenomenon of the quenching of the BChl fluorescence yield due to singlet–singlet annihilation in photosynthetic bacteria was studied by Campillo et al. [14], who used several mutants of *Rps. sphaeroides*. They concluded that the time-integrated fluorescence yield observed with all the reaction centers in the closed state can be fairly well described by an equation originally suggested by Swenberg et al. [15]:

$$\phi_f(z) = \phi_f^c \frac{r}{z} \log\left(1 + \frac{z}{r}\right) \tag{2}$$

where ϕ_f^c is the low intensity fluorescence yield observed with all the reaction centers closed, r is the ratio of the mono- to the biexcitation decay rate constant and z is the average number of excitations generated in a domain. Eqn. 2 is the special case for $r \to \infty$ of a more general expression derived by Paillotin et al. [16]. From the fact that Eqn. 2 gives a satisfactory fit to the experimental data for several purple bacteria Campillo et al. [14] concluded that in these photosynthetic systems there are large domains of several connected photosynthetic units, in which the rate of loss and trapping may easily exceed the rate of singlet–singlet annihilation [16]. This supports the conclusion of Vredenberg and Duysens [2]. From the experimental data no attempt was made to estimate the parameter r and thus no information about λ was obtained. More recently, experiments were done with a number of light-harvesting antenna complexes of several photosynthetic bacteria (containing no reaction centers), in which the total fluorescence yield was measured as a function of the pulse intensity, and the sizes of these complexes were estimated [17].

In Ref. 1, using a random-walk model [5] to describe the excitation transfer in a domain, we calculated the parameter r and found that it is a complicated function of λ and N, the number of antenna molecules per reaction center, the rate constants k_h, k_t^o, k_t^c and the rate constants for loss and biexcitation annihilation on an antenna, k_l and k_a, respectively (Eqn. 15 of Ref. 1). Using a Pauli master equation to describe the decay of the excitations in a domain, we further derived expressions for the time-integrated probability of loss per excitation, $U_\lambda(z)$, and the fraction of reaction centers closed, $V_\lambda(z)$, following a picosecond light pulse. In this case the reaction centers are all open before the pulse arrives and are converted into the closed state upon capturing an excitation. Since both $U_\lambda(z)$ and $V_\lambda(z)$ depend sensitively on the

parameters λ, N, k_l, k_h, k_t^o, k_t^c and k_a, their measurement leads to accurate estimates of these parameters, as we shall see in the sequel.

2. Materials and Methods

Chromatophores of *R. rubrum* and *Rps. capsulata* were prepared as described elsewhere [18]. For the experiments in which the time-integrated fluorescence induced by a picosecond laser pulse was measured, a home-built spectrofluorimeter was used. The excitation pulse was generated by a mode-locked Nd-YAG oscillator, amplified and frequency-doubled to 532 nm. The final pulse had a maximum energy of 6 mJ and a width at half height of 35 ps. The laser pulse hit a scattering device placed at 2 cm from the cuvette to produce a homogeneous excitation profile. The homogeneity of the beam profile was checked with a TV-camera and intensity fluctuations of less than 20% over the illuminated part of the cuvette were measured. Similar results were obtained with a photograph of the beam profile. Earlier experiments in which BChl light-harvesting complexes of several photosynthetic bacteria were studied with the same experimental arrangement yielded estimates of complex sizes that compared well with those obtained by other methods [17]. The illuminated surface of the cuvette was 0.25 cm^2, the optical pathlength 0.5 cm. All samples had a transmission higher than 90% at 532 nm to provide all parts of the sample with the same light intensity.

The fluorescence passed through a monochromator and was detected by a photodiode (RCA C 30810). The monochromator was shielded from the scattered laser light by a Schott KV 550 filter. A small fraction of the laser pulse was reflected on a second scattering surface, after which it was detected by a second calibrated photodiode to produce accurate energy measurements. The cuvette could be illuminated by a xenon flash (16 μs width at half height) or by a continuous beam. The xenon flash could be used to probe the low intensity fluorescence yield of the sample under several conditions, for example shortly before or after the laser pulse. The continuous light, which was used to maintain a certain fraction of reaction centers in the closed state, could be switched on at any time before the arrival of the pulse. Also, the xenon flash could be fired at any desired time before or after the pulse.

The absorption measurements were performed with a split beam spectrometer as described elsewhere [19]. The emission and excitation spectra of the preparations were recorded with a spectrofluorimeter [18]. In all experiments, the shape of the emission spectrum was independent of the pulse intensity. All experiments were done at room temperature.

3. *Rhodospirillum rubrum*

3.1. Experimental results

Fig. 1 shows the dependence of the inverse of the low intensity total fluorescence yield, ϕ_f, measured at 900 nm, on the fraction of closed reaction centers $\bar{x}$ in chromatophores of the purple bacterium *R. rubrum*. The data yield a straight line described by the reciprocal of Eqn. 1:

$$\phi_f^o/\phi_f = 1 - p\bar{x}, \quad p \approx 0.68 \tag{3}$$

The value of p varied somewhat from preparation

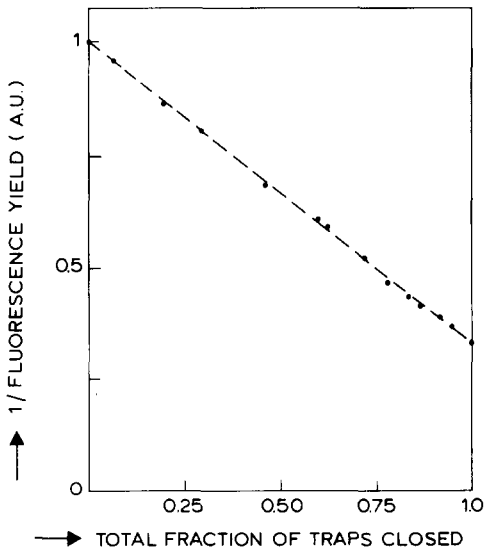

Fig. 1. Plot of the inverse of the low intensity fluorescence yield $1/\phi_f$ versus the macroscopic fraction $\bar{x}$ of reaction centers in the closed state observed in *R. rubrum* chromatophores. The fluorescence was detected at 900 nm, the fraction of reaction centers in the closed state was measured by the accumulation of the state $P^+ Q_1$ as indicated by the increase in absorption at 785 nm. The dashed line is the fit to the experimental data with $p = 0.68$ in Eqn. 1.

to preparation, but in all cases it was found that $0.67 \leq p \leq 0.72$. Thus we are led to the conclusion that in these chromatophores several photosynthetic units are connected to form a domain and in the following we shall try to find an estimate of their number, λ. The absolute fluorescence yield ϕ_f^o was found to be between 0.5% and 1% and the quantum efficiency for the charge separation $1-\phi_f^o$ to be approx. 95%, in agreement with earlier estimates ([20]; Kingma, H., personal communication). The fluorescence, ϕ_f, represents a fraction k_f/k_1 of the loss ϕ_1, where k_f is the rate of fluorescence on an antenna.

As mentioned in the introduction, Eqn. 3 has previously been interpreted to mean not only that several photosynthetic units are connected, but also that $k_h \gg k_t^o, k_t^c$ [3]. However, theoretical calculations of ϕ_f as a function of $\bar{x}$ [1], as well as computer simulations have made it clear that this interpretation is not fully justified. An almost identical dependence of ϕ_f on $\bar{x}$ was found for the two extreme cases $k_h \gg k_t^o, k_t^c$ and $k_h \ll k_t^o, k_t^c$ (the error in Eqn. 3 being less than 2% in both cases). Therefore, the fact that Eqn. 3 is observed in an experiment implies only that $\lambda \gg 1$, and in the following we shall try to find realistic estimates of the rate constants.

Using the same chromatophore preparation, we first measured the time-integrated fluorescence yield as a function of the pulse intensity I with all the reaction centers in the closed state before the arrival of the pulse. To close the reaction centers, 10 s of continuous background illumination was applied. That more than 95% of the reaction centers were indeed in the state P^+Q_1 (or $P^+Q_1^-$) was checked either using the weak xenon flash before the laser pulse, to monitor the fluorescence yield of the preparation, or measuring the transmission

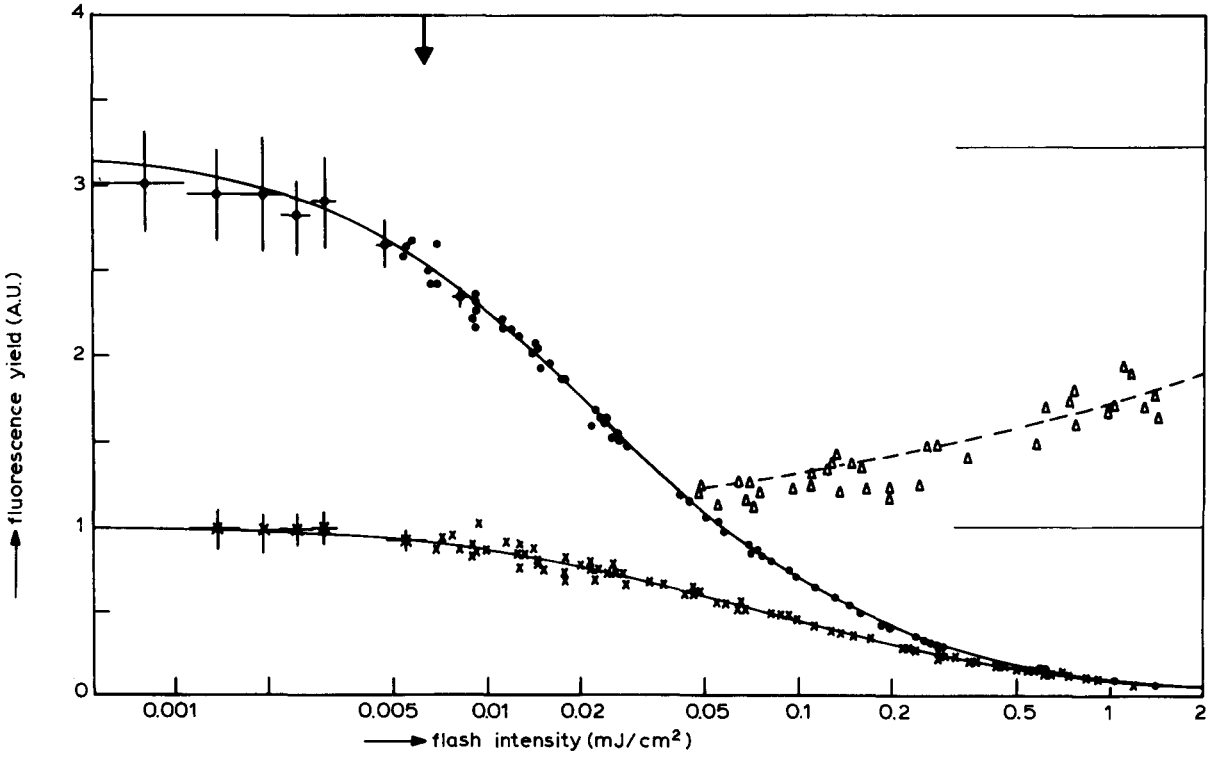

Fig. 2. Plots of the time-integrated fluorescence yield observed after a picosecond laser pulse as a function of the pulse intensity in *R. rubrum* chromatophores (●) with all the reaction centers kept in the closed state by continuous background illumination, (×) with all the reaction centers open before the pulse. The fluorescence yield detected with the weak xenon flash 1 ms after the laser pulse in the case where the reaction centers are initially all open is shown by the open triangles (△). The drawn lines represent the theoretical fits using Eqn. 22a and b in Ref. 1 with $\lambda = 16$, $\bar{k}_t^o = 19k_1$, $\bar{k}_t^c = 5.2k_1$ and $\bar{k}_a = 11.2k_1$, as described in the text. The arrow indicates the intensity of the laser flash where there is on the average one excitation per domain.

change at 880 nm, induced by the continuous illumination, in a separate experiment. The result is shown in Fig. 2 (●). One excited BChl per reaction center was obtained at $I = 0.125$ mJ/cm^2, taking one reaction center per 50 B880-BChl molecules (i.e., $N = 50$) [21], 31% for the efficiency of energy transfer of excitations (absorbed at 532 nm) to the network of B880-BChl [22] and an extinction coefficient of 140 $mM^{-1} \cdot cm^{-1}$ for B880-BChl at 880 nm [23]. It was found that the data for the fluorescence yield could be fitted to the theoretical expression derived by Paillotin et al. (see Ref. 16, Eqn. 20; see also Ref. 1, Eqn. 3) for r is approx. 1.0 (the best fit obtained with $r = 1.1$ is shown in Fig. 2).

Next we measured the time-integrated fluorescence yield with all the reaction centers open before the pulse. The conditions were such that more than 95% of the reaction centers were in the open state, PQ_1, before the pulse arrived. A dark period of 2 min between the successive pulses was sufficient for the excited reaction centers to relax to their dark state. Fig. 2 (×) shows the fluorescence yield as a function of the pulse intensity found under these conditions. It may be observed that a drop of the fluorescence yield below the low-intensity level occurs at higher intensities, compared to the case with all the reaction centers closed before the pulse. This effect is due to the transition of the reaction center. First, the effective number of excitations available for the annihilation process is reduced due to the fact that the open reaction centers act as stronger quenchers. Second, the closing of the reaction centers in the course of the transfer process results in an increasing probability of fluorescence for the remaining excitations. A maximum in the fluorescence yield does not occur. Note that at high pulse intensities the two curves in Fig. 2 tend to merge.

Finally, we measured the time-integrated fraction of reaction centers closed with all the reaction centers again open before the pulse. This was done in the following way. After the laser flash a certain fraction of the reaction centers is converted from the open state (PQ_1) into the closed state (P^+Q_1 or $P^+Q_1^-$), which in these chromatophores is stable for several tens of milliseconds at least. The fluorescence yield observed by exciting the sample subsequently with the weak xenon flash, 1 ms after the laser flash, monitors this fraction. The results we obtained as a function of the pulse intensity are shown in Fig. 2 (△). Using these data and those in Fig. 1 the fraction of closed reaction centers can be calculated. Note that even with the strongest flashes used, which generated more than five excitations per reaction center, only 65% of the reaction centers were closed.

3.2. Analysis of the experiments

The procedure followed to compare the experimental data with the theoretical formalism discussed in the preceding paper is as follows. The effective rates of trapping $\bar{k}_t^o$ and $\bar{k}_t^c$ (Ref. 1, Eqn. 6) are obtained from Fig. 1. Since the absolute quantum yield for the charge separation is about 95% we have $\phi_1^o \approx 0.05$. From the observed increase in the fluorescence yield upon closure of the reaction centers we find $\phi_1^c \approx 0.16$. For the loss rate constant k_1 we choose $k_1 = 5 \cdot 10^8$ s^{-1}, which corresponds to a fluorescence lifetime of 2 ns and a fluorescence quantum yield of about 10% if no reaction centers are present in the system [14,18]. Since $\phi_1^{o,c} = k_1/(k_1 + \bar{k}_t^{o,c})$ it follows that:

$$\bar{k}_t^o \approx 19 k_1 = 9.5 \cdot 10^9 \text{ s}^{-1}$$

$$\bar{k}_t^c \approx 5.2 k_1 = 2.6 \cdot 10^9 \text{ s}^{-1}$$

Since $r\ (= 2(k_1 + \bar{k}_t^c)/\bar{k}_a)$ is about 1.1 it further follows that:

$$\bar{k}_a \approx 11.2 k_1 = 5.6 \cdot 10^9 \text{ s}^{-1}$$

Now that we have established the effective rate constants $\bar{k}_t^o$, $\bar{k}_t^c$ and $\bar{k}_a$ we can continue to find λ or N_D, the number of antenna molecules per domain. This can be done in three ways. First we have fitted the experimental data for the total fluorescence yield and the fraction of reaction centers closed, observed with all the reaction centers initially open, to the functions $U_\lambda(z)$ and $V_\lambda(z)$ given by Eqns. 21 and 22 in ref. 1. Because for this system r is obtained quite accurately, the relation between the pulse intensity I and the average number of excitations per domain z can be found [16] (i.e., the ratio I/z) and the fits can be well done. This implies that a precise energy calibration and an estimate of the carotenoid to BChl energy transfer efficiency is not required.

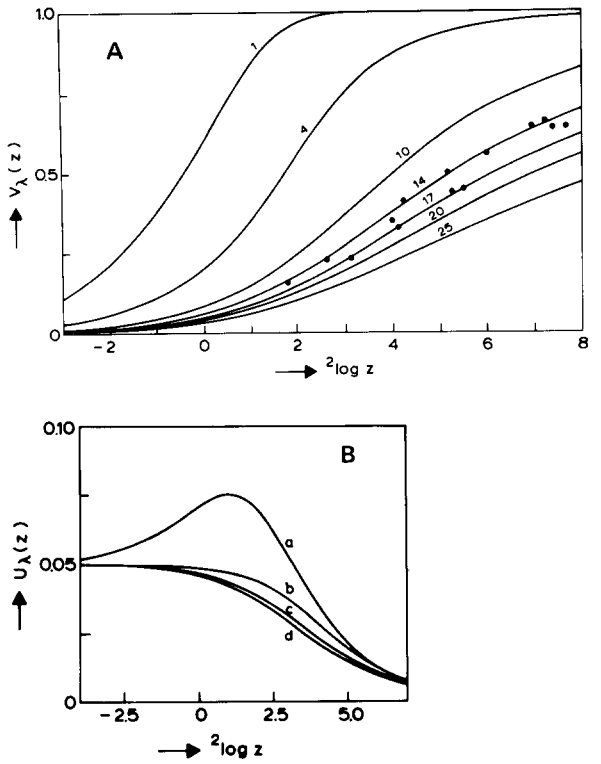

Fig. 3. (A). Plots of the total fraction of reaction centers closed in *R. rubrum* chromatophores as a function of the average number of excitations created per domain. The closed circles were calculated from the data in Fig. 2 (△) using Eqn. 1. The drawn curves are plots of the function $V_\lambda(z)$ (Eqn. 22b, Ref. 1) for $\bar{k}_t^o = 19k_1$, $\bar{k}_t^c = 5.2k_1$, $\bar{k}_a = 11.2k_1$ and several values of λ, as indicated with each curve shown in the figure. (B) Sketch of the function $U_\lambda(z)$ (Eqn. 22a, Ref. 1) for $\bar{k}_t^o = 19k_1$, $\bar{k}_t^c = 5.2k_1$, $\bar{k}_a = 11.2k_1$ and (a) λ = 1, (b) λ = 4, (c) λ = 10 and (d) λ = 25.

For a complex system this has the further advantage that no assumptions need to be made concerning the distribution of the excitations upon illumination. Fig. 3A shows the total fraction of reaction centers closed as a function of z. The data of Figs. 1 and 2 are used here. For several values of λ, using the numbers for $\bar{k}_t^o$, $\bar{k}_t^c$ and $\bar{k}_a$ given above, we have calculated the function $V_\lambda(z)$ given by Eqn. 22b in Ref. 1 and the results are plotted in Fig. 3A. It can be seen that the fractions of reaction centers closed, measured for a number of pulse intensities, all fall between the curves for λ = 14 and 17. Fig. 3B for the same set of λ values shows the total probability of loss per excitation $U_\lambda(z)$ given by Eqn. 22a in Ref. 1. Note that the curves for the fraction of reaction centers closed are distinguishable up to large values of λ, whereas those for the fluorescence tend to merge for λ ≥ 10. The curves for λ = 16 are drawn in Fig. 2 and present the best fit to the data.

Next, using the values for $\bar{k}_t^o$ and $\bar{k}_t^c$ given above we have fitted the functions $U_\lambda(z)$, $V_\lambda(z)$ and $U_o(z)$ to the three experimental curves shown in Fig. 2 by varying $\bar{k}_a$ and λ and using a computer optimalization procedure. This led to very similar results and we find $\bar{k}_a \approx 10.5k_1$ and λ ≈ 14. The value of 14–17 reaction centers in a domain corresponds to a domain size of 700–850 B880-BChl molecules [21].

Finally, the value of r obtained from the fluorescence data (r is approx. 1.1) can be used directly to find the relation between the pulse intensity I and z and determine the size N_D of the domain, as was done by Paillotin et al. [16] for Photosystem II of green plants. Using a carotenoid to B880-BChl energy transfer efficiency of 31% (see Ref. 22 and Bakker, J.G.C. and Kramer, H.J.M., unpublished observations) and a B880-BChl extinction coefficient of 140 $mM^{-1} \cdot cm^{-1}$ at 880 nm [23], we find the number of connected B880-BChl molecules in a domain to be between 800 and 1000, which corresponds to λ = 16–20 reaction centers per domain.

The agreement between the results obtained from the different approaches is excellent and the small differences may easily be due, for example, to uncertainty in the energy calibration. Note that the parameters λ and $\bar{k}_a$ can in principle be estimated without first determining the parameter r. However, the main reason why we find such accurate estimates for λ and $\bar{k}_a$ in the case of *R. rubrum* chromatophores is that the value of r can be so well determined. Small values of r or λ are nice for high accuracy.

3.3 The random-walk model

So far no assumptions are necessary concerning the details of the decay processes on the antenna molecules and the reaction center. In Ref. 1 we showed that the overall rate constants $\bar{k}_t^o$, $\bar{k}_t^c$ and $\bar{k}_a$ can be related to the microscopic rate constants k_t^o, k_t^c, k_a and k_h through a random-walk model, if N and λ are known. For this it is necessary to make some assumptions about the structure of the

network formed by the antenna molecules and about the dynamical behavior of the excitations in a domain. This is discussed in detail in Ref. 1. In the random-walk model the domain is represented by a regular square lattice of identical antenna molecules with regularly spaced traps. Since the antenna pigment system is constructed of small (8–10 kD), more or less regularly arranged transmembrane polypeptides, each containing one B880-BChl molecule, this seems a reasonable approximation [24,25]. The results that we obtain are not very sensitive to the particular choice of a square lattice. Different lattice types give approx. similar results (a different structure function would be needed in Ref. 1, Eqn. 9, but, for example, in Eqn. 15 this change would have a minor effect). The domain is assumed to be constructed of fully connected photosynthetic units. If the units would be connected via a few lattice points only the results would certainly be very different, but this would lead to a serious distortion of the hyperbolic relation (1) which is not observed.

For each microscopic rate constant a range of combinations with k_h can be chosen to yield the corresponding overall rate constant. The fit of the three overall rate constants $\bar{k}_t^o$, $\bar{k}_t^c$ and $\bar{k}_a$ simultaneously places a strong constraint on the final result. This is shown in Fig. 4, where those possible combinations of k_h, k_t^o, k_t^c and k_a are plotted that yield the values of $\bar{k}_t^o$, $\bar{k}_t^c$ and $\bar{k}_a$ given earlier (with $\lambda = 16$, $N_D = 800$). The results lead to the conclusion that:

$$k_h \geq 10^{12}\ s^{-1}$$

$$k_t^o = (4\text{–}6)\cdot 10^{11}\ s^{-1}$$

$$k_t^c \approx 1.4\cdot 10^{11}\ s^{-1}$$

$$k_a \geq 5\cdot 10^{12}\ s^{-1}$$

If $k_a \gg k_h$ is assumed, or perfect annihilation upon a collision of two excitations [26], it follows that:

$$k_h = (1\text{–}2)\cdot 10^{12}\ s^{-1}$$

It is not possible to estimate an upper bound for k_a from the experiments.

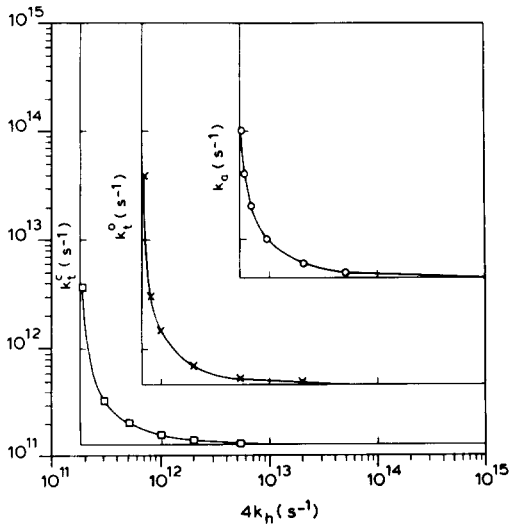

Fig. 4. Plots of the various possible combinations of the rate constants k_t^o, k_t^c, k_a and k_h, with $\lambda = 16$, $N = 50$ and $k_l = 5\cdot 10^8$ s^{-1}, that yield the overall rate constants $\bar{k}_t^o = 9.5\cdot 10^9$ s^{-1}, $\bar{k}_t^c = 2.6\cdot 10^9$ s^{-1} and $\bar{k}_a = 5.6\cdot 10^9$ s^{-1} obtained from the experiments (Figs. 1 and 2) for *R. rubrum* chromatophores.

4. *Rhodopseudomonas capsulata*

In the purple bacterium *Rps. capsulata* the fluorescence emission spectrum is known to have contributions from B850-BChl and B880-BChl, the B880-BChl fluorescence being the dominant part [27,28]. The extra emission observed after closure of the reaction centers comes from the B880-BChl [4,27,28]. The fluorescence quenching by carotenoid triplet states occurs mainly via the B880-BChl excited state [8]. These findings suggest that after the light pulse a major part of the excitations is rapidly transferred to the B880 antenna system, which then functions as the network connecting the reaction centers. We shall therefore apply the formalism of the preceding paper to the experimental data for the B880-BChl fluorescence yield.

Fig. 5 shows the total fluorescence yield observed with all the reaction centers initially open (□) and closed (●), respectively, as a function of the pulse intensity. Also the total fluorescence yield measured after the weak xenon flash, fired 1 ms after the laser pulse, is shown, which monitors the fraction of reaction centers closed. For extremely low pulse intensities the fluorescence yield

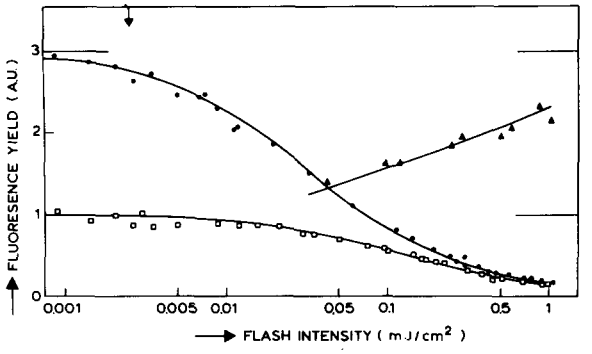

Fig. 5. Plots of the time-integrated fluorescence yield observed in chromatophores of *Rps. capsulata* after a picosecond laser pulse as a function of the pulse intensity (●) with all the reaction centers closed by continuous background illumination, (□) with all the reaction centers initially open. The fluorescence yield detected with the weak xenon flash 1 ms after the laser pulse in case the reaction centers are all open is shown by the closed triangles (▲). The drawn lines represent the theoretical fits using Eqns. 22a and b in Ref. 1 with $\lambda = 30$, $\bar{k}_t^o = 19k_1$, $\bar{k}_t^c = 5.7k_1$ and $\bar{k}_a = 2.7k_1$, as described in the text. The arrow indicates the intensity of the laser flash where there is on the average one excitation per domain.

at 900 nm increases by about a factor 3 upon the transition of the reaction centers. At the highest pulse intensities used about 80% of the reaction centers are closed. From the data for the total fluorescence yield in case the reaction centers are in the closed state (P^+Q_1) before the pulse unfortunately no precise value of r can be extracted and it can only be safely concluded that $r \geq 5$. For large values of r the curves are much alike. The data in Fig. 5 for the total fluorescence yield and the total fraction of reaction centers closed with the reaction centers initially open (PQ_1) are fitted for $\lambda = 30$ (with $r = 5$) and:

$$\bar{k}_t^o = 19k_1$$

$$\bar{k}_t^c = 5.7k_1$$

$$\bar{k}_a = 2.7k_1$$

For the latter we take the quantum efficiency of the primary photochemical process to be about 95%, similar to that observed for *R. rubrum* [20]. Most likely the additional losses in the B800/B850 antenna system are small and therefore we neglect them. The value $\lambda = 30$ corresponds to a domain size $N_D \approx 1000$–1100 B880-BChl molecules [29–31]. Similar values for the overall rate constants are obtained also from a computer optimalization procedure. Larger values of λ combined with larger values of r cannot be excluded, although from the fits it can be concluded that $20 \leq \lambda \leq 50$.

Using the random-walk model and taking $k_1 = 5 \cdot 10^8\ s^{-1}$, $k_a \gg k_h$ and $N = 35$, we find:

$$k_h \approx 4 \cdot 10^{11}\ s^{-1}$$

$$k_t^o \approx 3 \cdot 10^{11}\ s^{-1}$$

$$k_t^c \approx 1 \cdot 10^{11}\ s^{-1}$$

5. Discussion

This work describes a series of experiments in which the decay of excitations, generated in the antenna pigments of purple bacteria by a strong picosecond light flash, is studied. The fact that the relative contributions of the various decay channels, such as trapping and singlet–singlet annihilation, depend on the pulse intensity can be exploited to obtain information about the domain size and the microscopic rate constants on the antenna.

5.1. Rhodospirillum rubrum

The number of connected photosynthetic units forming a domain in *R. rubrum* chromatophores is between 10 and 20 and probably close to 16. This number may correspond to a large part of the reaction centers associated with a single chromatophore [19,31]. Preliminary experiments with whole cells indicate a similar number. Thus our experiments show that most of the B880-BChl molecules in a chromatophore form a network of antenna pigment molecules, connecting the reaction centers, among which efficient energy transfer takes place. The number of connected reaction centers is large enough to obtain Eqn. 1, as observed by Vredenberg and Duysens [2] and in this work. The rate of energy transfer between two neighboring B880-BChl molecules is $(1–2) \cdot 10^{12}\ s^{-1}$. This value, or rather the total transfer rate, does not strongly

depend on the type of lattice chosen. In the random-walk model it is assumed that the transfer takes place between nearest-neighbor antenna molecules only. A more realistic model is to allow also the transfer to other antennae and to choose the well-known R^{-6} dependence for the transition rates between the antenna molecules [32,33], where R is the distance between the excited and the unexcited molecule. This choice only slightly affects the k_h value. In the Förster theory for resonance dipole–dipole energy transfer [32,33], a standard distance R_0 is defined through the equation $k_h = \tau_0^{-1}(R_0/R)^6$, in which τ_0 is the natural radiative lifetime of the excited molecule (for BChl $\tau_0 \approx 18$ ns [34]) and R_0^6 depends on the overlap integral between the emission spectrum of the excited molecule and the absorption spectrum of the unexcited molecule, as well as on a parameter that describes the orientation of the two transition moments and the vector that connects them. With $R_0 = 90$ Å for a random orientation of the B880-BChl transition moments in a plane [14,35] this yields $R = 13$–15 Å. This distance is significantly shorter than that found by Campillo et al. [14]. It is of the same order as the B800/B850 dipole–dipole distance in the B800/B850 light-harvesting complex of *Rps. sphaeroides* [18].

For this particular system we find a rate of trapping $k_t^o = (4\text{–}5) \cdot 10^{11}$ s^{-1}. A microscopic trapping time of 2–3 ps may correspond to the reduction time of the reaction center bacteriopheophytin (Bph) to the state P^+Bph^- [36]. This state may therefore act as a closed trap for the remaining excitations in the antennae. The captured excitations can escape by a recombination process [37–39] to the surrounding B880–BChl molecules, but this happens probably at a rate of 1–2 ns^{-1} [39,40]. The escape probability from an excited open reaction center back to the antennae is 90% or more. Therefore, the conclusion originally drawn from Eqn. 1 by Duysens is correct for this case.

We have neglected the formation of triplet states in the antenna system that act as possible traps for the excitations. In *R. rubrum*, upon 532 nm excitation, a large part of the absorbed excitations decays directly into a carotenoid triplet state (see Ref. 41 and also Kingma, H., manuscript in preparation); however, our experiments can be satisfactorily explained without taking this into account. One may think of several reasons to explain the absence of noticeable effects of carotenoid triplet states. First, the carotenoid triplet state may not be formed on a ps time scale. Second, it may not be a quencher of the B880-BChl excited state immediately after its formation (see, for example, Refs. 13 and 42), although it certainly is one on a ns or μs timescale ([41]; Van Bochove, A.C. and Van Grondelle, R., unpublished observation). Third, it may be that the presence of a few carotenoid triplet states does not lead to a significant decay channel. This needs further investigation and a possible way to proceed is to study explicitly the competition between the quenching by carotenoid triplet states and that due to singlet–singlet annihilation, e.g., by forming a given amount of triplet states before the picosecond laser flash, preferably in a system without reaction centers.

5.2. Rhodopseudomonas capsulata

The number of connected photosynthetic units in a domain of chromatophores of *Rps. capsulata* is larger than 20 and probably around 30. For such large domains, the estimate of r is rather inaccurate and only a lower bound is obtained. However, the fact that we find some 80% of the reaction centers closed at the highest pulse intensity used ensures that λ is smaller than 50. The value $\lambda = 30$ may again correspond to the number of reaction centers in a single chromatophore of *Rps. capsulata* [31] and this once more indicates that all the reaction centers in a chromatophore are connected via the B880-BChl. The rate of energy transfer between two neighboring B880-BChl molecules is estimated to be about $4 \cdot 10^{11}$ s^{-1}. This would correspond to $R \approx 17$ Å (if again $R_0 = 90$ Å and $\tau_0 = 18$ ns). The somewhat larger distance in this species may reflect the fact that the antenna system of *Rps. capsulata* at some places accommodates the B800/B850 light-harvesting complexes, resulting in a somewhat lower rate of excitation transfer.

Our results support the hypothesis that most of the singlet–singlet annihilation takes place in the B880-BChl. However, annihilation in the B800/B850 light-harvesting antennae is known to occur [17] and therefore a complete analysis of these phenomena should include the B800/B850 an-

tenna as a separate entity. Experiments at 77 K, where back-transfer from B880 to B800/B850 does not occur, may be well suited for such an analysis.

The calculated rate of trapping by an open reaction center, $k_t^o \approx 3 \cdot 10^{11}$ s^{-1}, is very close to that obtained for *R. rubrum*, indicating that the same reaction, i.e., the formation of the state P^+Bph^-, is responsible for the closing of the reaction center.

6. Concluding remarks

The results of the analysis of the experimental data for both species depend relatively strong on the estimate of the absolute quantum yield of photosynthesis observed when the reaction centers are all open. It is therefore important that this yield is established accurately. For example, if the quantum yield would have been below 80%, instead of 95% as used here, this would have seriously affected the calculated parameters.

Recently, it was reported that the antenna of purple bacteria contains a special long-wavelength pigment [43,44], absorbing around 905 nm, that is supposed to connect the normal B880-BChl antenna molecules to the reaction center. It was suggested that the excitations concentrate on this special pigment. For an explanation of our experimental results we do not require such a hypothesis. In fact, it may be in conflict with our interpretation of the fluorescence data. If a special long-wavelength pigment would indeed form the connection between the reaction centers and the B880-BChl network, the system would behave as one with separated units, certainly at temperatures below 150 K, a behavior which we found not to occur (Van Grondelle, R. and Rijgersberg, C.P., unpublished observations). Moreover, even at room temperature the excitations would be rapidly concentrated on these special pigments, which would probably give rise to a completely different annihilation profile than that observed.

If picosecond laser pulses are applied to measure fluorescence lifetimes, either with a streak camera or by measuring absorbance changes, it is not sufficient, in general, to use pulse intensities of about one excitation per reaction center in order to prevent annihilation occurring. For example, in the case of *R. rubrum* this would correspond to an average of $z = 10-20$ excitations per domain and in that case the singlet–singlet annihilation is the dominant decay process during the first part of the decay, as may be observed from our experimental data. Therefore, to obtain fluorescence lifetimes that reflect only the trapping and loss processes in a photosynthetic system much lower pulse intensities should be used.

In conclusion we may say that this work shows that the combined measurement of the total fluorescence yield and the total fraction of reaction centers closed after an intense picosecond light pulse, as a function of the pulse intensity, is well suited to study the microscopic structure of the domain. The application of the random-walk model appears to lead to sensible estimates of parameters that so far had not been obtained with sufficient accuracy.

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