Biochimica et Biophysica Acta, 1015 (1990) 295–303
Elsevier

BBABIO 43123

Competition between energy trapping and exciton annihilation in the lake model of the photosynthetic membrane of purple bacteria

J. Deprez [1], G. Paillotin [1], A. Dobek [1,2], W. Leibl [3,*], H.-W. Trissl [3] and J. Breton [1]

[1] *Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette (France),* [2] *Institute of Physics, A. Mickiewicz University, Poznan (Poland) and* [3] *F.B. Biologie / Chemie, Biophysik, Universität Osnabrück, Osnabrück (F.R.G.)*

(Received 23 May 1989)
(Revised manuscript received 19 September 1989)

Key words: Energy transfer; Exciton annihilation; Photosynthesis; Purple bacterium

General equations are formulated to describe the competition between primary photochemistry (trapping) by photosynthetic reaction centers (RCs) and bimolecular singlet-singlet annihilation which occur upon excitation with picosecond laser flashes. These equations are solved under the assumption of fast equilibration and free energy migration between photosynthetic units (lake model) with homogeneous antenna and of irreversible trapping (the initial charge separation does not repopulate an excited state). The yield and the kinetics of fluorescence and of trapping are calculated as a function of the energy of the picosecond flash and of the fraction of RCs closed before excitation. Theoretical curves are adjusted to fluorescence yield measurements in the purple bacterium *Rhodospirillum rubrum* (Bakker, J.C.G., Van Grondelle, R. and Den Hollander, W.T.F. (1983) Biochim. Biophys. Acta 725, 508–518) and are used to analyse time-resolved photovoltage measured on *Rhodobacter sphaeroides* R26.1 whole cells. From these analyses, a parameter that relates the competition between trapping and annihilation as well as the ratio of the quenching efficiency for open and closed RCs are determined.

Introduction

Upon excitation of photosynthetic membranes by very short laser flashes, the decay of the excitation involves two main processes: trapping and losses. Here trapping shall be defined as the formation of the first charge separated state. The losses can be divided into monomolecular (such as fluorescence and intersystem crossing) and bimolecular (such as singlet-singlet annihilation) processes.

In the reaction center (RC) of purple bacteria, three essential molecules are involved in electron transport during the first redox reactions: P, the primary electron donor, H, the intermediary acceptor and Q_A, the first stable electron acceptor. Excitation of isolated RCs in the redox state PHQ_A gives rise to an excited state P^*HQ_A which initiates, in about 3 ps, the primary charge separation $P^+H^-Q_A$ before the electron is transferred from H to Q_A on a 100–300 ps time-scale [1–3].

In the photosynthetic membrane, the migration and trapping processes occur in the subnanosecond time range. Thus, even in the case of excitation by picosecond flashes, the concentrations of RCs in the different states PHQ_A, P^*HQ_A, $P^+H^-Q_A$ and $P^+HQ_A^-$ vary during the lifetime of the excitons in the antenna. The reduction of P^+ and the oxidation of Q_A^- by secondary electron carriers take place on a time-scale longer than 10 ns [4–6] and the concentration of the RCs in a state resulting from these secondary reactions may thus be considered to be constant during the exciton lifetime.

The appearance of the states $P^+H^-Q_A$ and $P^+HQ_A^-$ has been detected by time-resolved absorption [7] and light-gradient photovoltage measurements [8]. On the other hand, the fluorescence decay kinetics monitor the lifetime of the exciton in the antenna [9,10].

In this work, we analyze the subnanosecond processes resulting from the excitation by a picosecond flash of the photosynthetic membrane of purple bacteria in which the concentration of two states of the RCs is

Abbreviations: RC, reaction center; PSU, photosynthetic unit; P, primary donor; H, intermediary pheophytin acceptor; Q_A, first quinone acceptor.

* Present address: CEN Saclay.

Correspondence: J. Breton, Service de Biophysique, Département de Biologie, CEN de Saclay F-91191 Gif-sur-Yvette Cedex, France.

List of terms used

α	parameter relating the competition between annihilation and trapping ($\alpha = \gamma/2 \cdot \Gamma \cdot k_o$)
E	energy of excitation flash (photons·cm^{-2})
f	proportionality factor in the light-gradient theory
Φ	fluorescence yield
γ	bimolecular singlet-singlet annihilation rate constant
Γ	quantum yield of primary photosynthetic charge separation
$I(t)$	intensity of excitation flash (photons·cm^{-2}·s^{-1}) (photon flux)
k_i	rate constants
$n(t)$	mean number of excitons per RC
N	antenna size/size of PSU (average number of antenna pigments per RC)
$q_o(t)$	fraction of open RCs
Q_o	fraction of open RCs before the flash
q_{of}	fraction of open RCs after the flash
$q_c(t)$	fraction of closed RCs
σ	absorption cross section of one mean antenna pigment
T_{eff}	transmission of one membrane of an effective vesicle
τ_i	time constants
V	photovoltage
V_0	photovoltage created by closure of all RCs in a single membrane
z	normalized excitation in hits per trap ($z = \sigma \cdot N \cdot E$)

varied before excitation. These two states, which have different quenching rate constants [11,12], are defined as:

(i) the 'open' state, PHQ_A, in which the RC is able to perform photochemistry. The trapping of an exciton by an RC in this state is defined by the appearance of the charge separated state $P^+H^-Q_A$;

(ii) the 'closed' state, in which the primary donor is oxidized (P^+). This state exhibits a lifetime much longer than the exciton lifetime [4–6].

In the photosynthetic membrane, a statistical photosynthetic unit (PSU) is defined as the ratio of antenna pigments to RCs. In addition, the excitons created in a given PSU may not stay confined within this unit but can migrate to neighboring units. These connected PSUs constitute a photosynthetic domain which, in the case of purple bacteria, has been shown to be large [13–15]. In many photosynthetic purple bacteria the light-harvesting system is heterogeneous, consisting of peripheral and core antenna. Since the latter lies at lower energy, the excitation is very quickly transferred from the peripheral to the core antenna where it gets equilibrated and trapped by the RC [12,16]. It has been proposed that the connectivity occurs at the level of the core antenna [14].

At low intensity, when no more than one exciton is present per domain, the excitons decay by the usual monomolecular processes, characterized by the rate constants k_o and k_c for open and closed RCs, respectively:

$$k_o = k_{1o} + k_f + k_g \quad (1)$$

$$k_c = k_{1c} + k_f + k_g \quad (2)$$

where k_{1o} denotes the rate constant of trapping, k_{1c} the quenching rate constant of P^+, k_f the decay rate constant by fluorescence and k_g the rate constants of all other loss processes.

At high intensity, when several excitons are created simultaneously in a domain, bimolecular exciton-exciton annihilation can occur in addition to the other decay pathways [12,13,17]. Upon excitation with picosecond flashes, the major bimolecular deactivation process is by singlet-singlet annihilation [17], which may lead to the disappearance of either one (Eqn. 3) or both (Eqn. 4) excitons:

$$S_1 + S_1 \xrightarrow{\gamma_1} S_1 + S_0 \quad (3)$$

$$S_1 + S_1 \xrightarrow{\gamma_2} S_0 + S_0 \quad (4)$$

where γ_1 and γ_2 denote the corresponding bimolecular rate constants.

The detailed mechanisms of energy transfer have been considered by several authors, including Knox [18,19]. Pearlstein [20] and Paillotin [21] and were summarized by Van Grondelle [12]. Several theories have been derived to determine the trapping time for photosynthetic systems in the low energy limit [22–24]. Furthermore, several theoretical descriptions of the dependence of the fluorescence yield on the energy of a picosecond flash when exciton-exciton annihilation is present have been proposed. For example, Paillotin et al. have formulated a Master equation approach to extract the size of photosynthetic domains [25,26]. The theory is limited to the case where the RC is either in the closed state or altogether absent. Den Hollander et al. [27] also used a Master equation to derive the fraction of RCs closed as a function of the average number of excitons per domain when all RCs are in the open state before the flash.

None of these theories covers the kinetics of fluorescence and trapping as well as the case where a fraction of RCs is in the closed state before the flash. However, time-resolved photovoltage measurements have recently been shown to provide valuable information on the trapping time, both in the low and high intensity regime, as well as on the trapping yield when a fraction of RCs is in the closed state before the flash [8,28].

In this work, assuming fast equilibration and free migration of the excitons between PSUs in a large domain (lake model), a combination of analytical and numerical treatment of the equations describing exciton

trapping and annihilation in the photosynthetic membrane after picosecond excitation is presented. The yields and the kinetics of fluorescence and trapping are calculated as a function of the intensity of the picosecond excitation flash and of the initial concentration of open reaction centers. These calculated curves are compared to a set of experimental data obtained by fluorescence and photovoltage measurements in purple bacteria.

Theoretical approach

Trapping and excitation decay: general equations in the lake model

We consider a domain consisting of many RCs (lake model) in which the average number of antenna pigments per RC is N. In this case, the time dependence of the mean number, $n(t)$, of excitons per RC and the fractions, $q_o(t)$ and $q_c(t)$, of RCs in the open and closed states when the domain is excited by an incident photon flux $I(t)$ may be expressed [25,26] using the general Eqns. 5 to 7, in which σ is the absorption cross-section per molecule of pigment in the antenna, $\gamma = \gamma_1 + 2 \cdot \gamma_2$ is the overall bimolecular decay rate constant according to Eqns. 3 and 4 and Γ is the quantum yield of formation of the closed state (P^+) when all the RCs are in the open state [25].

$$\frac{dn(t)}{dt} = \sigma N I(t) - k_o n(t) q_o(t) - k_c n(t) q_c(t) - \tfrac{1}{2}\gamma n(t)^2 \quad (5)$$

$$\frac{dq_o(t)}{dt} = -\Gamma k_o n(t) q_o(t) \quad (6)$$

$$\frac{dq_c(t)}{dt} = \Gamma k_o n(t) q_o(t) \quad (7)$$

For delta function excitation, $I(t) = E \cdot \delta(t)$, the exciton-generating term $\sigma \cdot N \cdot I(t)$ in Eqn. 5 can be set equal to zero, and

$$\frac{dn(t)}{dt} = -n(t)[(k_o - k_c) q_o(t) + k_c] - \tfrac{1}{2}\gamma n(t)^2 \quad (8)$$

In the following, we define z as the initial number of excitons created per RC ($z = n(0) = \sigma \cdot N \cdot E$) and we introduce the dimensionless parameter $\alpha = \gamma/2 \cdot \Gamma \cdot k_o$ which measures the competition between singlet-singlet annihilation and exciton capture by open RCs. We also consider that only a fraction of RCs, Q_o, is open before the flash. Then, Eqns. 8 and 6 give:

$$\frac{d}{dt}\left(\frac{q_o(t)}{Q_o}\right) = -\left(\frac{q_o(t)}{Q_o}\right)^{\alpha+1}\left[\Gamma k_o Z + Q_o \frac{k_o - k_c}{\alpha - 1} + \frac{k_c}{\alpha}\right] + \left(\frac{q_o(t)}{Q_o}\right)^2 Q_o \frac{k_o - k_c}{\alpha - 1} + \left(\frac{q_o(t)}{Q_o}\right)\frac{k_c}{\alpha} \quad (9)$$

and:

$$n(t) = -\frac{1}{\Gamma k_o}\left(\frac{q_o(t)}{Q_o}\right)^{-1}\frac{d}{dt}\left(\frac{q_o(t)}{Q_o}\right) \quad (10)$$

After the complete decay of the excitons in the antenna, $d(q_o(t)/Q_o)/dt = 0$. The fraction of RCs still open reaches its final value, q_{of}, which can be detemined from the following Eqn. 11, deduced from Eqn. 9 in stationary state:

$$\left(\frac{q_{of}}{Q_o}\right)^{\alpha}\left[\Gamma k_o Z + Q_o \frac{k_o - k_c}{\alpha - 1} + \frac{k_c}{\alpha}\right] = \left(\frac{q_{of}}{Q_o}\right) Q_o \frac{k_o - k_c}{\alpha - 1} + \frac{k_c}{\alpha} \quad (11)$$

In the absence of annihilation, the extrapolation of Eqn. 11 for $\alpha \to 0$ gives:

$$\Gamma \frac{k_o}{k_c} z = q_{of}\left(1 - \frac{k_o}{k_c}\right) - \ln\frac{q_{of}}{Q_o} + Q_o\left(\frac{k_o}{k_c} - 1\right) \quad (11a)$$

It can be shown by numerical solution of Eqns. 5 to 7 and by comparison with the value of q_{of} deduced from Eqn. 11a that this equation contains the special case of longer excitation flashes (nanosecond laser flashes) in which singlet-singlet annihilation can be neglected. In addition for $k_o = k_c$, Eqn. 11a simplifies to:

$$\frac{q_{of}}{Q_o} = e^{-\Gamma z} \quad (11b)$$

which is the exponential saturation law derived from the cumulative Poisson statistic in a separate unit model [29].

The fluorescence yield, Φ, is defined by:

$$\Phi = \frac{k_f}{z}\int_0^\infty n(t)\,dt \quad (12)$$

Integrating Eqn. 6 gives the relationship between Φ and the residual fraction, q_{of}, of open RCs after excitation:

$$\Phi = -\frac{k_f}{\Gamma k_o z}\ln\left(\frac{q_{of}}{Q_o}\right) \quad (13)$$

Prediction for the yields and kinetics of trapping and fluorescence

Trapping yield. Eqn. 11 can be used to calculate the residual fraction, q_{of}, of open RCs after excitation as a function of the energy of the picosecond flash (parameter z), of the initial concentration of open RCs (parameter Q_o), of the competition between exciton-exciton annihilation in the antenna and trapping by open RCs (parameter α), and of the relative quenching efficiencies of the RCs in the open and closed states (parameter k_o/k_c).

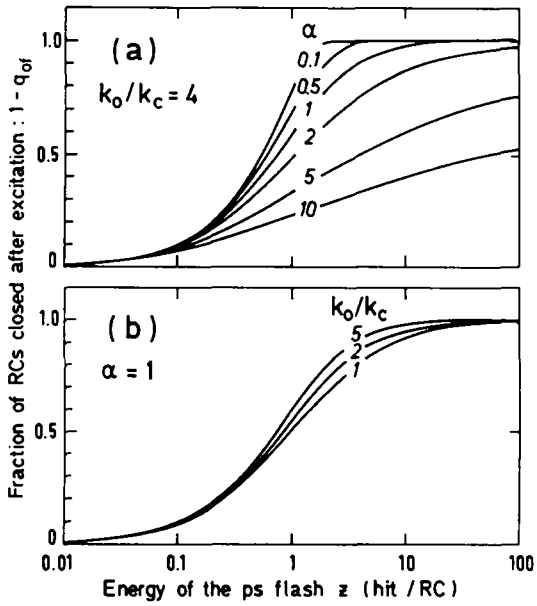

Fig. 1. Fraction of RCs closed after excitation by a picosecond flash, $(1 - q_{of})$, as a function of the average number of photons absorbed per RC, z. All the RCs are in the open state before excitation ($Q_o = 1$). The quantum yield of primary charge separation is assumed to be $\Gamma = 1$. (a) Influence of the competition between exciton-exciton annihilation and trapping. Large values of α indicate a strong annihilation. For all curves, $k_o/k_c = 4$. (b) Influence of the ratio of the quenching rate constants for the open and closed states of the RCs ($\alpha = 1$).

When all the RCs are open before excitation ($Q_o = 1$), the fraction $(1 - q_{of})$ of RCs closed by the picosecond excitation is plotted as a function of z in Fig. 1. For a given excitation intensity, $(1 - q_{of})$ increases when α decreases and when k_o/k_c increases, i.e., when annihilation and exciton capture by RCs in the closed state compete less efficiently with trapping by open RCs.

When only a fraction Q_o of the RCs is open before excitation, the fraction of RCs closed by the picosecond excitation $(Q_o - q_{of})$ can also be calculated from Eqn.

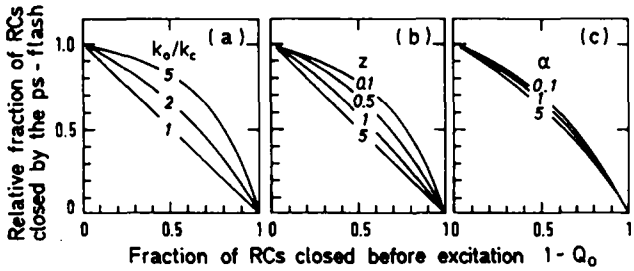

Fig. 2. Relative fraction of RCs closed by a picosecond flash as a function of the initial fraction of RCs in the closed state, $(1 - Q_o)$. The curves are normalized on the fraction of RCs closed by the picosecond flash when all the RCs are open before excitation. $\Gamma = 1$. (a) Influence of the ratio of the quenching rate constants for the open and closed states, k_o/k_c ($z = 0.1$, $\alpha = 0.01$). (b) Influence of the average number of photons absorbed per RC, z ($k_o/k_c = 4$, $\alpha = 0.01$). (c) Influence of α, describing the competition between annihilation and trapping ($k_o/k_c = 4$, $z = 0.5$).

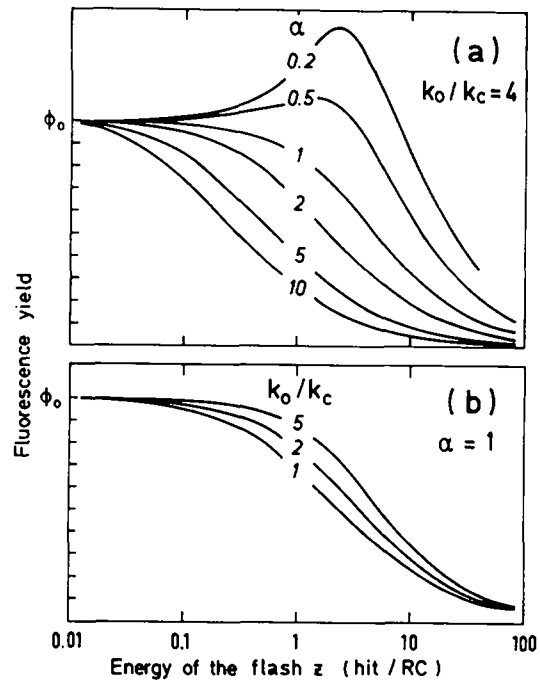

Fig. 3. Time-integrated fluorescence yield Φ measured on a picosecond flash excitation as a function of the average number of photons absorbed per RC. All the RCs are in the open state before excitation ($Q_o = 1$). The curves are normalized to the fluorescence yield Φ_o calculated in the low energy limit ($z \to 0$). $\Gamma = 1$. (a) Influence of the competition between annihilation and trapping. Large values of α indicate a strong annihilation ($k_o/k_c = 4$). (b) Influence of the ratio of the quenching rate constants for the open and closed states, k_o/k_c ($\alpha = 1$).

11. For presentation, this quantity is normalized to the fraction of RCs that would be closed by the flash if all the RCs were open before excitation (Fig. 2). When all other parameters are held constant, a progressive increase of the fraction of closed RCs causes a decrease in the fraction of RCs closed by the picosecond excitation, but this decrease is less pronounced than predicted by a proportionality to Q_o. This nonlinearity is due to the fact that the closed RCs quench the excitons less efficiently. The higher the value of k_o/k_c, the higher is the probability of excitons being trapped by open RCs and the more pronounced is the nonlinearity (Fig. 2a). This corresponds to an increase of the apparent absorption cross-section of still open RCs. The effect is more pronounced when the exciton density is low ($z \ll 1$) and tends to disappear at higher exciton density ($z > 1$) (Fig. 2b). In the latter case the quenching efficiency of closed RCs has little influence on the closure of the remaining open RCs.

When annihilation is important (α increasing) the overall fraction of RCs closed after excitation decreases (Fig. 2c). This process is reflected in Fig. 2c only by the losses induced in the population of excitons migrating from PSUs with closed RCs towards PSUs with open RCs and thus appears as a second-order effect.

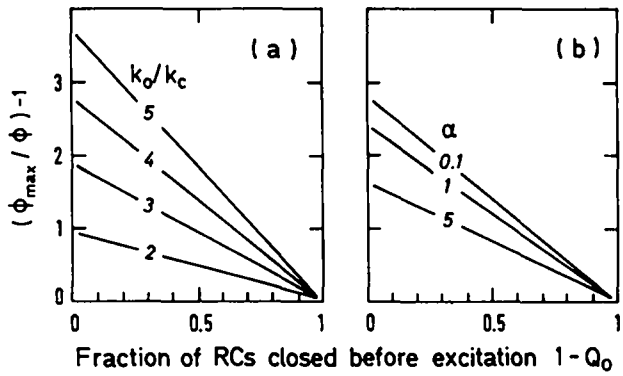

Fig. 4. Stern-Volmer plots of the fluorescence yield induction as a function of the initial fraction of closed RCs, $(1-Q_o)$. Φ is the fluorescence yield calculated for the picosecond flash and Φ_{max} is the value of Φ calculated when all the RCs are closed before excitation ($Q_o = 0$). $\Gamma = 1$. (a) Influence of the ratio of the quenching rate constants for the open and closed states, k_o/k_c. (b) Influence of α, describing the competition between annihilation and trapping.

Fluorescence yield. The integrated fluorescence yield, Φ, can be calculated according to Eqn. 13 in which the residual fraction of open RCs, q_{of}, after the excitation flash is determined from Eqn. 11.

The dependence of the fluorescence yield, Φ, on the excitation energy, z, when all the RCs are open before excitation is plotted in Fig. 3. Φ is normalized to the value Φ_0 calculated in the low energy limit ($z \to 0$) for which annihilation can be neglected.

When bimolecular annihilation does not compete with exciton trapping ($\alpha \ll 1$), the fraction of RCs converted to the closed state increases with z (see Fig. 1a). If the quenching efficiency of the closed state is lower than that of the open state ($k_c < k_o$), this induces an increase in the fluorescence yield. The higher k_o/k_c, the more pronounced is this induction phenomenon (Fig. 3a) which takes place during the lifetime of the excitons.

At higher energy, or for large values of α, the fluorescence induction phenomena get masked by bimolecular annihilation processes (Fig. 3a). The quenching of the fluorescence upon increasing z is less pronounced when k_o/k_c is large (Fig. 3b).

The progressive closure of a fraction $(1 - Q_o)$ of the RCs gives rise to classical induction phenomena. Stern-Volmer plots of $(\Phi_{max}/\Phi) - 1$ as a function of $(1-Q_o)$ are shown in Fig. 4, where Φ_{max} is the fluorescence yield calculated for a picosecond probe flash when all the RCs are closed before excitation ($Q_o = 0$). The fluorescence induction increases with k_o/k_c (Fig. 4a) and is attenuated when bimolecular annihilation processes introduce losses during excitation transfer between PSUs (Fig. 4b).

Kinetics of trapping and fluorescence. The time dependence of the fraction of open RCs, q_o, can be obtained by numerically solving Eqn. 9. The calculated value $q_o(t)$ can then be used in Eqn. 10 to determine the kinetics of the decay of the exciton concentration, $n(t)$, in the antenna.

A set of trapping kinetics and exciton decay curves, corresponding to different values of α and for a given value of the other parameters, is depicted in Fig. 5. Singlet-singlet annihilation causes an apparent acceleration of trapping (Fig. 5a), due to a shortening of the exciton lifetime in the antenna (Fig. 5b), in addition to the decrease of the efficiency of closure of the RCs already noticed (Fig. 1a).

Although the exciton decay is usually not exponential, we will denote in the following by τ_f the time needed for $n(t)$ to reach the value z/e and by τ_o the time for which the fraction $(Q_o - q_o(t))$ of RCs closed is equal to $(Q_o - q_{of})(1 - e^{-1})$. In the low energy limit, the values of τ_f and τ_o are equal to k_o^{-1} if all RCs are open before excitation.

The dependence of τ_o and τ_f on the excitation intensity is shown in Fig. 6 for different values of α. It is interesting to note that the trapping time is less affected by the bimolecular annihilation processes than the exciton lifetime and starts to be independent of α when there are three or more excitons created per RC. In the absence of annihilation, the shortening of the trapping time at high energies is due to the bimolecular interaction between the excitons and the traps.

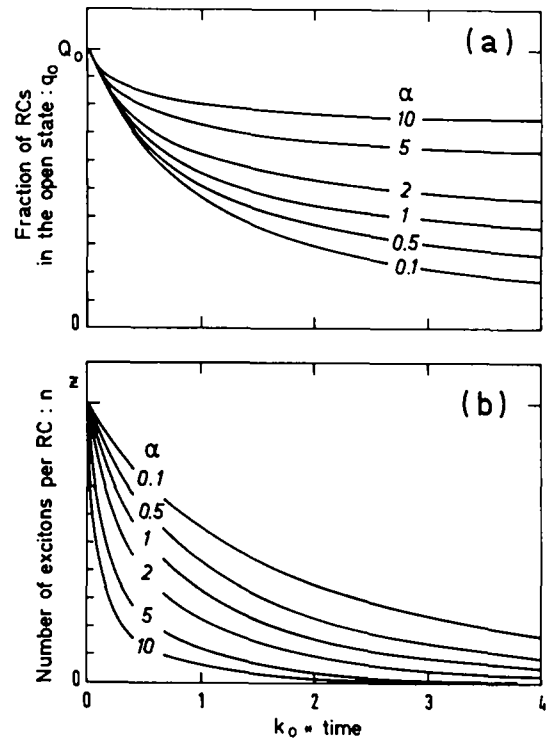

Fig. 5. Profiles of the decay (a) of the fraction of RCs in the open state, q_o and (b) of the average number of excitons per RC, n. Influence of α, describing the competition between annihilation and trapping. For all curves, the average number of photons absorbed per RC is $z = 1$, the initial fraction of open RCs is $Q_o = 0.5$, the ratio of the quenching rate constants of the open and closed states is $k_o/k_c = 4$ and $\Gamma = 1$.

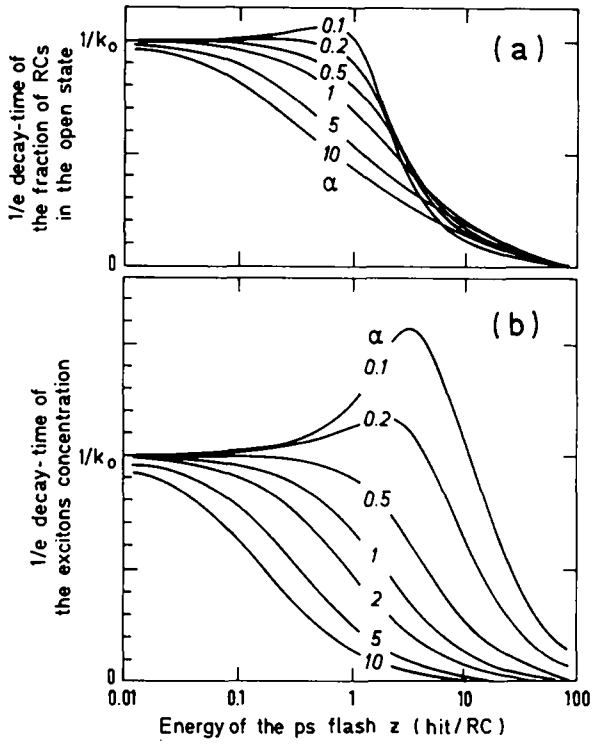

Fig. 6. Decay times (a) of the fraction of RCs in the open state, q_o, (b) of the average number of excitons per RC, n, as a function of the average number of photons absorbed per RC, z. All the RCs are in the open state before excitation. Influence of α, describing the competition between annihilation and trapping. For all curves, the ratio of the quenching rate constants for the open and closed states is $k_o/k_c = 4$ and $\Gamma = 1$.

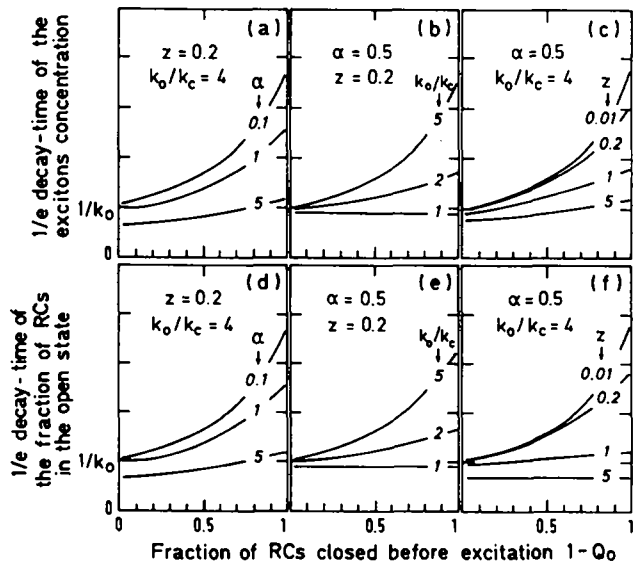

Fig. 7. Decay times (a, b, c) of the average number of excitons per RC, n, and (d, e, f) of the fraction of RCs in the open state, q_o, as a function of the initial fraction of closed RCs, $(1 - Q_o)$. $\Gamma = 1$. (a, d) Influence of α, describing the competition between annihilation and trapping. (b, e) Influence of the ratio of the quenching rate constants for the open and closed states, k_o/k_c. (c, f) Influence of the average number of photons absorbed per RC, z.

The dependence of τ_o and τ_f on the fraction $(1\text{-}Q_o)$ of RCs closed before excitation is shown in Fig. 7. The classical fluorescence induction already noted (Fig. 4) is due to the increased exciton lifetime when a fraction of RCs is closed and $k_o > k_c$. The fluorescence decays faster with stronger annihilation (Fig. 7a), or with more efficient quenching of the closed state (Fig. 7b), or with increasing excitation energy (Fig. 7c). All these dependencies correspond to a decrease in the fluorescence yield, as already discussed (Figs. 3 and 4). The trapping time also gets faster either with stronger annihilation (Fig. 7d), or with more efficient quenching of the closed state (Fig. 7e), or with increasing excitation energy (Fig. 7f).

For the case of pronounced energy transfer between PSUs ($k_o/k_c = 4$) and rather low energy ($z = 0.2$), the kinetics of fluorescence and trapping get slower with increasing fraction of closed RCs (Figs. 7a and 7d). The increase of annihilation ($\alpha = 0.1$ to 5) masks this lengthening. The kinetics of fluorescence and trapping are affected in a very similar way by the closure of the RCs (compare Figs. 7a and 7d). The decrease in the quenching efficiency by closed RCs also induces a parallel lengthening of the fluorescence decay and of the trapping kinetics (Figs. 7b and 7e).

A comparison of Figs. 7c and 7f demonstrates a different behaviour of the trapping and exciton kinetics in different ranges of energy. In the low energy limit ($z = 0.01$), the decay of $q_o(t)$ follows the exciton decay $n(t)$. This is no longer the case at higher energy ($z \approx 1$). At high energy, especially if a considerable fraction of RCs is already closed, the remaining open RCs become closed before the untrapped excitons have disappeared. Thus, the trapping time can be much faster than the fluorescence lifetime. This trend is less pronounced when the effect of annihilation is larger.

To our knowledge, this comparison of fluorescence lifetimes and trapping kinetics, in both the low and high energy range, has never been treated before. The present theory allows the molecular parameters that govern the exciton dynamics to be extracted from measurements of both fluorescence and photovoltage not only in the low energy limit but also in the presence of annihilation.

Comparison with experimental data

In the following, the applicability of the theory will be demonstrated by an analysis of fluorescence yield measurements reported in *Rhodospirillum rubrum* [15] and of new photovoltage data on *Rhodobacter sphaeroides* R26.1. The fluorescence yield measurements in Ref. 15 have been previously analyzed by the theory of Den Hollander et al. [27]. This allows a comparison of the theoretical approaches to be made. A treatment

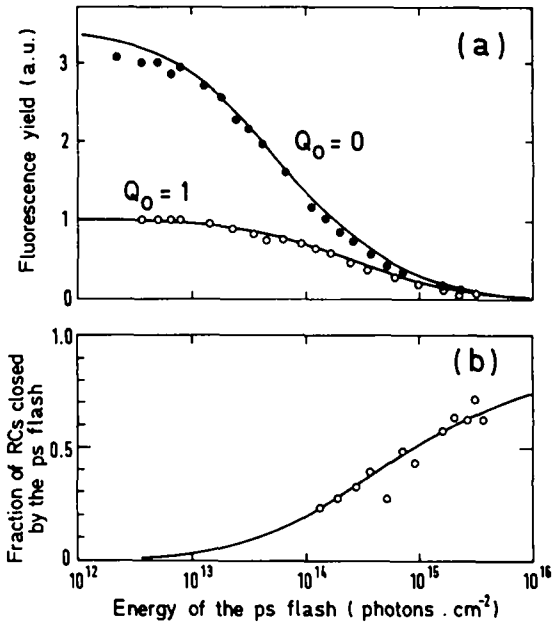

Fig. 8. (a) Energy dependence of the time-integrated fluorescence yield Φ measured (Fig. 2 in Ref. 15) upon excitation of *R. rubrum* chromatophores by a 35 ps, 532 nm laser flash. ●, all the RCs were closed before excitation ($Q_o = 0$) by background illumination. ○, all the RCs were open before excitation ($Q_o = 1$). (b) Fraction, q_{of}, of RCs closed by the picosecond flash (from Figs. 1 and 2 in Ref. 15) when all the RCs were open before excitation. Continuous lines in (a) and (b) are calculated from Eqns. 11 and 13 with $\Gamma = 0.95$, $k_o/k_c = 3.4$, $\alpha = 4$ and $N \cdot \sigma = 3 \cdot 10^{-15}$ cm^2.

of a more extended set of photovoltage data, including kinetics, is given in the accompanying paper [30].

Analysis of fluorescence yield measurements

In *R. rubrum*, a 3- to 4-fold increase in the fluorescence yield is observed when the primary donor is converted into the oxidized state P^+ [9,10,15]. In Fig. 8a is plotted the time-integrated fluorescence yield Φ measured by Bakker et al. [15] as a function of the energy of a 532 nm ps flash in *R. rubrum* chromatophores, either with all the RCs kept in the closed state or with all the RCs open before the flash. For open RCs ($Q_o = 1$) Fig. 8b shows the fraction q_{of} of RCs closed by the picosecond flash. q_{of} is determined by the fluorescence yield measured with a weak xenon flash (Fig. 2 in Ref. 15) together with the calibration of this fluorescence yield by absorption change measurements (Fig. 1 in Ref. 15).

The continuous lines in Fig. 8 represent the theoretical fits using Eqns. 11 and 13 with $\Gamma = 0.95$, $k_o/k_c = 3.4$, $\alpha = 4$ and $N \cdot \sigma = 3 \cdot 10^{-15}$ cm^2. Taking, as in Ref. 15, an absorption cross-section $\sigma = 6 \cdot 10^{-17}$ cm^{-2} at 532 nm for the B880 molecules, the average number of antenna molecules per RC is $N = 50$. In Fig. 8b, even for the strongest excitation used, which generates more than five excitons per RC, only 65% of the RCs are closed. This strong competition between exciton-exciton annihilation and the capture of excitons by open RCs is reflected by the large value of α.

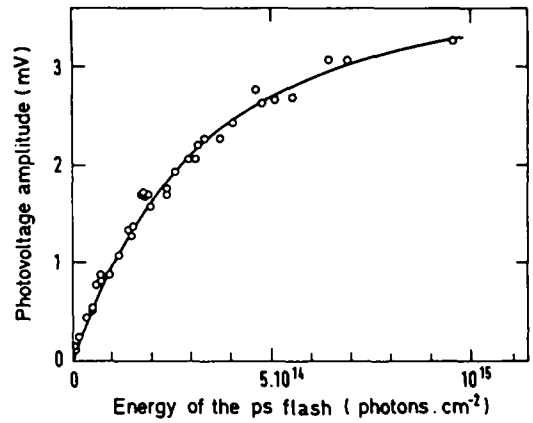

Fig. 9. Energy dependence of the amplitude of the photovoltage elicited from *Rb. sphaeroides* R26.1 whole cells by a 30 ps, 532 nm laser flash [30]. The continuous line is calculated from Eqn. 11 with $\Gamma = 0.95$, $k_o/k_c = 3.0$, $\alpha = 1$, $N \cdot \sigma = 3 \cdot 10^{-15}$ cm^2 and $f \cdot V_o = 4$ mV.

Both theories fit the data equally well, as seen by a comparison of the curves in our Fig. 8 and in Fig. 2 of Ref. 15. Using a similar value for the ratio k_o/k_c, the present theory allows for the annihilation parameter α to be extracted, whereas with the other theory the analogue parameter, r, is not accessible with sufficient precision. On the other hand, the theory of Den Hollander et al. [30] gives access to the number of units per domain, which in *R. rubrum* is found to be 16 ± 2.

Analysis of photovoltage measurements

In light-gradient experiments [8,31,32], the amplitude of the photovoltage elicited by a picosecond flash from a suspension of photosynthetic vesicles can be related to the fraction of RCs closed by the excitation [33].

The amplitude of the photovoltage from *Rb. sphaeroides* R26.1 is plotted in Fig. 9 as a function of the 532 nm picosecond flash energy [30]. In double flash experiments, the photovoltage signal was recorded on a

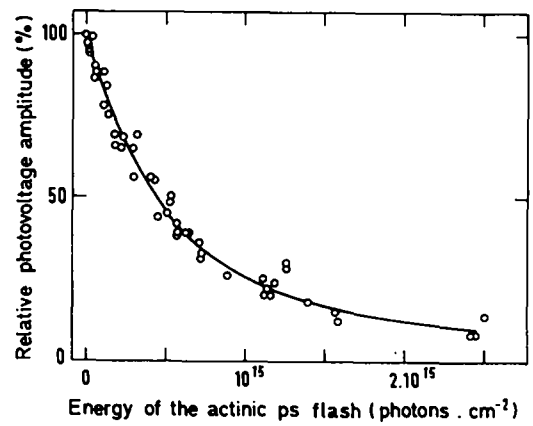

Fig. 10. Amplitude of the photovoltage elicited from *Rb. sphaeroides* R26.1 whole cells by a 30 ps, 532 nm laser probe flash, as a function of the energy of a 30 ps, 532 nm actinic flash preceding the probe flash by 20 ns [30]. The continuous line is calculated with the same set of parameters as for Fig. 9.

probe flash of given energy, which was applied 20 ns after an actinic flash of variable energy. The amplitude of the photovoltage elicited by the probe flash is a measure of the fraction Q_o of RCs still open after the actinic flash, and is expected to decrease when the energy of the actinic flash increases. The results of such experiments are depicted in Fig. 10, in which the amplitude of the photovoltage on the probe flash is normalized to its value in the absence of the actinic flash ($Q_o = 1$). The amplitude, V, of the photovoltage is correlated with the fraction of RCs closed by the picosecond excitation by Eqns. 14 and 14a in Ref. 33. Using these equations and Eqn. 5, the data in Figs. 9 and 10 are well fitted with $\Gamma = 0.95$, $\alpha = 1$, $k_o/k_c = 3.0$, $N \cdot \sigma = 3 \cdot 10^{-15}$ cm^2 and with the transmission coefficient T_{eff} and the maximum photovoltage amplitude $f \cdot V_0$ [33] equal to 0.003 and 4 mV, respectively. This set of parameters gives also a good fit of the photovoltage kinetics as a function of both the excitation energy and the initial fraction of open RCs [30]. The 4-times higher value of α in *R. rubrum* indicates that annihillation competes more strongly with trapping in this species than in *Rb. sphaeroides* R261. The competition appears to be also more pronounced in *R. rubrum* than in *Rb. capsulatus* [15].

Conclusion

A theory is developed which describes the competition between bimolecular annihilation and trapping in the photosynthetic membrane of purple bacteria upon picosecond flash excitation. Assuming a lake model in which two states of the RCs are characterized by their exciton quenching efficiencies, the differential equations describing the annihilation processes and the fluorescence and trapping kinetics can be solved. Comparison of calculated curves with experimental data (see also Refs. 30,34) shows that this macroscopic picture of the energy transfer is accurate enough to describe the main features of trapping and fluorescence decay in photosynthetic purple bacteria. The main limitation of this theory is the assumption of a free energy transfer between PSUs. The finite dimension of the photosynthetic domain is taken into account in more detailed theories [25–27] in which the number of connected PSUs is one of the parameters attempted to be determined. These theoretical approaches are certainly more realistic in describing the excitation transfer but they need a mathematical formalism which makes them hard to be intuitively understood.

Applying one of these theories [27] for chromatophores of *R. rubrum* and *Rb. capsulatus*, domain sizes of 16 ± 2 and 35 ± 15 are found, respectively [12,15]. The fluorescence yield starts to decline for energies of a picosecond flash lower than 10^{13} photons $\cdot$ cm^{-2} (Fig. 8a), which correspond to less than 0.03 photons absorbed per RC [15]. Since exciton-exciton annihilation can only occur if two or more excitons are created in the same domain, this suggests that more than 20 PSUs are connected in *R. rubrum* chromatophores. Fluorescence yield simulations in Ref. 26 have shown that the lake model is a good approximation when the number of connected units exceeds 10. Thus, although the approach used in this work in its present form does not give information about the photosynthetic domain size, its formulation is justified in the case of purple bacteria.

The simple formalism allows this theory to be easily used as a first analysis of the experimental data. The occurrence of significant deviations from this phenomenological description should then suggest to the experimentalist the need of a more elaborate theory to extract more information from the measurements.

Acknowledgements

The work at A. Mickiewicz University was in part supported by Research Project Nr: RP.H.13.1.10 to A.D.; W.L. and H.-W.T. acknowledge the financial support of the Deutsche Forschungsgemeinschaft (SFB 171).

References

1 Woodbury, N.W., Becker, M., Middendorf, D. and Parson, W.W. (1985) Biochemistry 24, 7516–7521.
2 Martin, J.L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA 83, 957–961.
3 Kirmaier, C. and Holten, D. (1987) Photosynth. Res. 13, 225–260.
4 Mathis, P. (1983) in The Biology of Photoreception (Cosens, D.J. and Vince-Price, D., eds.), pp. 223–248, Society for Experimental Biology, Great Britain.
5 Holten, D., Windsor, M.W., Parson, W.W. and Thornber, J.P. (1978) Biochim. Biophys. Acta 501, 112–126.
6 Dutton, P.L., Prince, R.C. and Tiede, D.M. (1978) Photochem. Photobiol. 28, 939–949.
7 Nuijs, A.M., Van Grondelle, R., Joppe, H.L.P., Van Bochove, A.C. and Duysens, L.N.M. (1985) Biochim. Biophys. Acta 810, 94–105.
8 Deprez, J., Trissl, H.-W. and Breton, J. (1986) Proc. Natl. Acad. Sci. USA 83, 1699–1703.
9 Sebban, P. and Moya, I. (1976) Biochim. Biophys. Acta 722, 436–442.
10 Borisov, A.Yu., Freiberg, A.M., Godik, V.I., Rebane, K.K. and Tipman, K.E. (1985) Biochim. Biophys. Acta 807, 221–229.
11 Wredenberg, W.J. and Duysens, L.N.M. (1963) Nature 197, 355–357.
12 Van Grondelle, R. (1985) Biochim. Biophys. Acta 811, 147–195.
13 Campillo, A.J., Hyer, R.C., Monger, T.G., Parson, W.W. and Shapiro, S.L. (1977) Proc. Natl. Acad. Sci. USA 74, 1997–2001.
14 Monger, T.G. and Parson, W.W. (1977) Biochim. Biophys. Acta 460, 393–407.
15 Bakker, J.C.G., Van Grondelle, R. and Den Hollander, W.T.F. (1983) Biochim. Biophys. Acta 725, 508–518.
16 Van Grondelle, R. and Sundström, V. (1988) in Photosynthetic Light-Harvesting Systems (Sheer, H. and Schneider, S., eds.), pp. 403–438, De Gruyter, Berlin.
17 Breton, J. and Geacintov, N.E. (1980) Biochim. Biophys. Acta 594, 1–32.

18 Knox, R.S. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 183–221, Academic Press, New York.
19 Knox, R.S. (1977) in Primary Processes of Photosynthesis, Vol. 2 (Barber, J., ed.), pp. 55–97, Elsevier, Amsterdam.
20 Pearlstein, R.M. (1982) in Photosynthethesis: Energy Conversion by Plants and Bacteria, Vol. 1 (Govindjee, ed.), pp. 293–329, Academic Press, New York.
21 Paillotin, G. (1972) J. Theor. Biol. 36, 223–235.
22 Montroll, E.W. (1969) J. Math. Phys. 10, 753–765.
23 Den Hollander, W.T.F. and Kasteleyn, P.W. (1982) Physica 112A, 523–543.
24 Kenkre, V.M. and Vong, Y.M. (1981) Phys. Rev. B23, 3748–3755.
25 Paillotin, G., Swenberg, C.E., Breton, J. and Geacintov, N.E. (1979) Biophys. J. 25, 513–534.
26 Paillotin, G., Geacintov, N.E. and Breton, J. (1983) Biophys. J. 44, 65–77.
27 Den Hollander, W.T.F., Bakker, J.C.G. and Van Grondelle, R. (1983) Biochim. Biophys. Acta 725, 492–507.
28 Trissl, H.-W., Leibl, W., Deprez, J., Dobek, A. and Breton, J. (1987) Biochim. Biophys. Acta 893, 320–332.
29 Mauzerall, D. (1982) in Biological Events Probed by Ultrafast Laser Spectroscopy (Alfano, R.R., ed.), pp. 215–235, Academic Press, New York.
30 Dobek, A., Deprez, J., Paillotin, G., Leibl, W., Trissl, H.-W. and Breton, J. (1990) Biochim. Biophys. Acta 1015, 313–321.
31 Trissl, H.-W. and Kunze, U. (1985) Biochim. Biophys. Acta 806, 136–144.
32 Trissl, H.-W. (1983) Proc. Natl. Acad. Sci. USA 80, 7173–7177.
33 Leibl, W. and Trissl, H.-W. (1990) Biochim. Biophys. Acta 1015, 304–312.
34 Trissl, H.-W., Breton, J., Deprez, J., Dobek, A. and Leibl, W. (1990) Biochim. Biophys. Acta 1015, 322–333.