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Minireview

Picosecond processes in chromatophores at various excitation intensities

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Abstract

The aim of this paper is to review and discuss the results obtained by fluorescence and absorption spectroscopy of bacterial chromatophores excited with picosecond pulses of varying power and intensity. It was inferred that spectral and kinetic characteristics depend essentially on the intensity, the repetition rate of the picosecond excitation pulses as well as on the optical density of the samples used. Taking the different experimental conditions properly into account, most of the discrepancies between the fluorescence and absorption measurements can be solved. At high pulse repetition rate ($> 10^6$ Hz), even at moderate excitation intensities (10^{10}–10^{11} photons/cm^2 per pulse), relatively long-lived triplet states start accumulating in the system. These are efficient (as compared to the reaction centers) quenchers of mobile singlet excitations due to singlet-triplet annihilation. The singlet-triplet annihilation rate constant in *Rhodospirillum rubrum* was determined to be equal to 10^{-9} cm^3 s^{-1}. At fluences $> 10^{12}$ photons/cm^2 per pulse singlet-singlet annihilation must be taken into account. Furthermore, in the case of high pulse repetition rates, triplet-triplet annihilation must be considered as well. From an analysis of experimental data it was inferred that the singlet-singlet annihilation process is probably migration-limited. If this is the case, one has to conclude that the rate of excitation decay in light-harvesting antenna at low pumping intensities is limited by the efficiency of excitation trapping by the reaction center. The influence of annihilation processes on spectral changes is also discussed as is the potential of a local heating caused by annihilation processes. The manifestation of spectral inhomogeneity of light-harvesting antenna in picosecond fluorescence and absorption kinetics is analyzed.

Abbreviations: LHA – light-harvesting antenna; RC – reaction center

1. Introduction

In experiments employing methods of picosecond spectroscopy to investigate primary processes in photosynthesis, it is almost always the case that excitation intensities are considerably higher than in natural photosynthesis. Recently, however, spectrometers with picosecond time resolution have been devised whose sensitivities allow for much low pulse excitation intensities on a level of $I \sim 10^6$ photons/cm^2 and lower (for a review, see Freiberg 1986, Holzwarth 1987). An important feature of such instruments is the high pulse repetition rate (up to 10^8 Hz). On one hand, the low-fluence excitation better simulates natural photosynthesis. On the other hand, the high pulse repetition rates must be considered when comparing the results with those obtained in single-pulse (low-frequency) picosecond experiments. In particular, in case of high-frequency excitation the fluorescence kinetics may be perturbed by the long-lived trip-

let excitations generated by the preceding light pulses (Breton et al. 1979, Geacintov and Breton 1977, Geacintov et al. 1978, Holzwarth 1987). The lifetime of triplet states, e.g., carotenoids in bacterial photosynthetic organisms, equals $\tau_T = 2 - 9$ μs (Rademaker and Hoff 1981). The quantum yield of the intersystem crossing can reach $\phi_{IC} = 0.3$ (Monger and Parson 1977, Rademaker and Hoff 1981, Kingma 1983). Based on these data the number of triplet excitations n_T can be estimated by the following steady-state formula:

$$n_T = \Phi_{IC} n_S \tau_T / \tau_\Delta , \quad (1)$$

where τ_Δ is the pulse repetition period and n_S the number of singlet excitations generated by each pulse. Thus, it can be seen that with high repetition rates ($\tau_\Delta = 12$ ns) the number of triplet excitations may exceed that of singlets by several orders of magnitude, i.e., $n_T \simeq 150\ n_S$ when $\Phi_{IC} = 0.3$ and $\tau_T = 5$ μs.

In picosecond experiments performed with single pulses, the relative number of triplet states can be lower by three orders of magnitude. However, since much higher fluences are generally used ($I \geqslant 10^{13}$photons/cm^2 per pulse), singlet-singlet annihilation processes become a significant excitation quenching process (for review, see Campillo and Shapiro 1978, Geacintov and Breton 1982, van Grondelle 1985).

In studies of excitation quenching kinetics, both fluorescence and absorption methods are used. In some cases, the results obtained by these two methods appear to be in conflict and are the basis for some divergence in views. At room temperature and at low excitation intensities the fluorescence decay kinetics in *Rhodospirillum (R.) rubrum* is a single-exponential throughout the entire emission band (Freiberg et al. 1984, 1987, 1989, Borisov et al. 1985, Godik et al. 1988), whereas the absorption methods suggest double-exponential decay kinetic when the reaction centers (RC) are initially closed (Sundström et al. 1986, van Grondelle 1987, Bergström et al. 1989, Visscher et al. 1989). Woodbury and Bittersmann (Woodbury and Bittersmann 1990) found a slight deviation from single-exponentiality in the fluorescence kinetics as well but this observation remains to fully explained. Note that in the intensity range greater than $I > 10^{13}$ photons/cm^2 per pulse, the inclusion of singlet-singlet annihilation processes has resulted in a successful solution of a number of seeming contradictions (Kudzmauskas et al. 1985, 1986, 1988, Valkunas 1986). In literature, there are also contradictory statements about the character of the excitation quenching obtained by the fluorescence and absorption method. Sundström et al.(1986), van Grondelle (1987), Bergström et al. (1989) and Visscher et al.

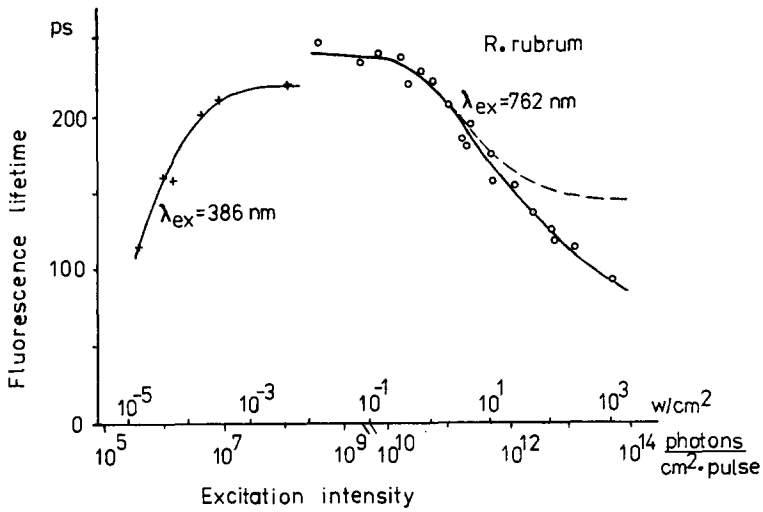

Fig. 1. Dependence of the average excitation lifetime (for definition see Freiberg et al. 1989) on intensity for bacterial chromatophores *R. rubrum* (two separate excitations). Pulse repetition rate 82 MHz, room temperature. Excitation wavelength 762 nm (○) or 386 nm (+); recording at 900 nm with resolution 4 nm (○) or spectrally nonselectively in the main fluorescence band (+). The dotted line denotes the result of theoretical calculations allowing for the processes of singlet- triplet annihilation.

(1989) maintain that the process of excitation quenching by RC is migration-limited. The same inference has been made by Danielius and Razjivin (1988). However, the analysis of the quantum yields of fluorescence (Bakker et al. 1983, van Grondelle 1985) and fluorescence kinetics (Freiberg et al. 1984, 1989) lead to an opposite conclusion: the quenching of excitation by RC is trapping-limited.

In order to clarify the situation and to resolve the noted inconsistencies, in the present communication a critical analysis of the results obtained by picosecond fluorescence and picosecond absorption methods, based on simple and transparent physical models, is given. Experimental data mainly for the bacteria *R. rubrum* characterized by the simplest light-harvesting antenna (LHA), have been used. We expect that the results of the analysis are, in principle, applicable also in case of a more complex photosynthetic organisms including higher plants. The excitation intensities employed differ by eight orders of magnitude, extending from the lowest excitation intensities close to natural photosynthesis, to very high fluences where singlet-singlet annihilation processes become important (see Fig. 1).

2. Decay kinetics at low excitation intensities

Minimum excitation intensities, with which picosecond excitation decay kinetics have been measured, is in range of 10^5–10^6 photons/cm^2 per pulse for fluorimetry (Freiberg et al. 1984, Borisov et al. 1985, Freiberg 1986) (see Fig. 1), and 10^{12}–10^{13} photons/cm^2 per pulse for differential absorption spectroscopy (Sundström et al. 1986, van Grondelle et al. 1987). As mentioned above, such low excitation intensities are obtained only because of the high pulse repetition rates ($> 10^6$ Hz). At room temperatures, the results of fluorimetry (Freiberg et al. 1984) indicate that, within experimental precision of the measurment, singly exponential decay of the excited states in *R. rubrum*, regardless of the recording wavelength and the state of RC. In case of photochemically active RC, the excitation lifetime in LHA is about 60 ps. With the increase of the excitation intensity, as the larger fraction of the RC becomes oxidized, the lifetime increases to 250 ps, but the kinetics remain exponential. At the same time, as measured by absorption methods, the decay curves are explicitly nonexponential (Sundström et al. 1986). At 77 K, the kinetics measured by both fluorescence (Freiberg et al. 1987, Godik et al. 1988) and absorption (van Grondelle et al. 1987) methods, become nonexponential. However, differences of decay constants in various spectral regions are observed. We note here that, in contrast to fluorescence measurements, in most of the absorption experiments the excitation and probing wavelengths coincide. On the assumption that in the region of low intensities, the annihilation processes can be ignored, the kinetics are associated mainly with the processes of excitation migration in LHA and capture by RC. In the next section, the experimental results are discussed from this point of view, whereby the problem of spectral inhomogeneity of the LHA is also considered.

3. Influence of spectral inhomogeneity of LHA and the excitation/recording wavelengths on decay kinetics

The simplest model describing the migration of excitation in LHA contains but one kinetic parameter, W, the rate of the jump between antenna pigment molecules. In a spectrally homogeneous LHA, this parameter is well defined by the excitation diffusion coefficient. In a spectrally inhomogeneous LHA, W becomes less well defined because of the appearance of a larger number of kinetic parameters describing the transfer between different spectral forms which depend in turn on the structure of the LHA. In general, the excitation decay kinetics are no longer single-exponential and this can provide information about the make up of the system. In order to discuss various experimental situations differing in their excitation and recording wavelengths, we will use the energy level scheme depicted in Fig. 2, which generalizes the commonly used LHA built up by two spectral forms or LHA of randomly distributed spectral forms and is liable to thorough and straightforward analysis. The set of energy levels in this scheme characterizes the possible spectral forms of

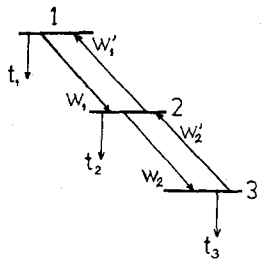

Fig. 2. Simplified energy level scheme, modelling of an inhomogeneous LHA in *R. rubrum*. W_i and W_i'-transfer rates between the corresponding levels, i-excitation lifetime in the ith level when there is no energy transfer between levels.

LHA. The quantities W_i and W_i' are the kinetic constants of the energy transfer between different spectral forms, τ_i the excitation lifetimes corresponding to these forms which are in turn influenced by certain other kinetic processes (natural excitation decay and its capture by RC). A numerical study of the excitation decay kinetics at various energy levels has been performed for two cases characteristic of the experiments discussed: (a) when excitation and recording proceeds on the same energy level, and (b) when a shorter wavelength absorbing state is excited, while recording occurs from a longer-wavelength absorption state. The numerical analysis shows that all the excitation decay kinetics can be qualitatively classified as follows:

1. When all τ_i values are close to one another, then, regardless of the values of W_i and W_i', as well as of the level excited, all the kinetics calculated are practically single-exponential (small deviation from single-exponential) and close to one another.
2. If one assumes that $\tau_1, \tau_2 \gg \tau_3$, then variations can be observed depending on the excitation used.

In the case (a) and with the assumption that $W_1 > W_2 > \tau_3^{-1}$, the kinetics can become nonexponential (see Fig. 3). The fast decay phase is conditioned by the decay of the excitation from the level excited, while the slow phase is connected with the overall decay throughout the system, that the decay constant is close to τ_3 (it is assumed that $W_i^{-1} < \tau_3$). Therefore, the contribution of the slow exponential is determined by the return of excitation from the lower levels to the upper levels (i.e., by the values of W_i'). By lowering the temperature, the values of the return rate, W_i', decrease and the fast exponential is more visible. Within the limit $W_i' \approx 0$, the kinetics become single-exponential and are determined by the rate of the excitation escape from the state under consideration, W_i.

In the case (b) and with the assumption that all W_i are of the same order and larger than τ_3^{-1}, the decay kinetics are essentially single-exponen-

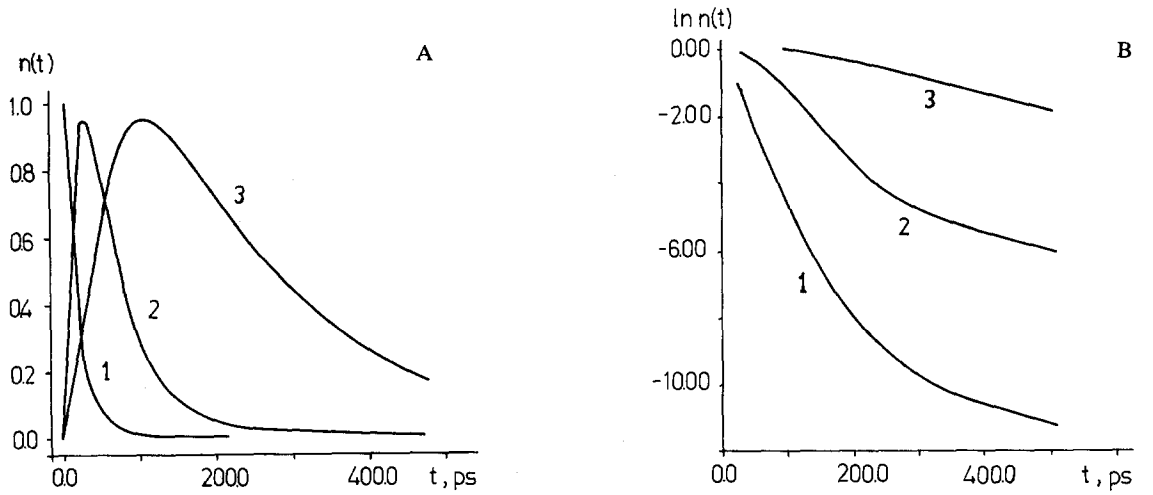

Fig. 3. Calculated kinetics of excitation decay, based on the scheme of Fig. 2. Initially excited is only level 1. Curves 1, 2, 3 correspond to the kinetics at levels 1, 2, 3, respectively. Parameters: $\tau_2 = 1$ ns; $1/(W_2') = 4$ ns; $1/W_2 = 40$ ps; $1/W_1 = 20$ ps; $1/W_1' = 2$ ns; $\tau_3 = 200$ ps; $\tau_1 = 2$ ns. In the part A, the kinetics are presented in linear scale, in the section B, in semilogarithmic scale.

tial and close to τ_3. A quick redistribution of excitations between the levels determines the initial growth of the excitation population at the level under consideration, which may result in the delay of the excitation decay kinetics. With the decrease of W_i' (i.e., with a lowering of the temperature) and with the assumption that $W_1^{-1} < W_2^{-1} < \tau_3$ the decay kinetics on the middle level of Fig. 2, becomes nonexponential with a fast component connected with W_2^{-1}, a slow one conditioned by τ_3 and a delay defined by W_1^{-1}. In the opposite case, if $\tau_3 > W_1^{-1} > W_2^{-1}$, the fast exponential of the incident part of the decay kinetics is damped by the rise of fluorescence with the characteristic time W_1^{-1}. The excitation decay kinetics at the lowest level of the model system under consideration is single-exponential with a delay time of $\approx W_1^{-1} + W_2^{-1}$.

All these qualitative dependencies were easily accomodated in the models developed for the numerical simulation of the experimental picosecond fluorescence kinetics of *Rhodobacter (Rb.) sphaeroides* and *Chr. minutissimum* (Freiberg et al. 1989) as well of *R. rubrum* (Freiberg et al.1990a).

Note that the case (a) is peculiar to absorption experiments, while the case (b), is characteristic of fluorescence experiments. Situation (1) represents the model of an inhomogeneous LHA, in which excitation is captured by RC with practically the same rates from all spectral forms. Situation (2) is an opposite model: the capture by RC occurs only from the longer-wavelength spectral form of LHA. The necessity of introducing at least three spectral forms into the model rather than only two, which is often the limit (Borisov et al. 1982, Kramer et al. 1984, van Grondelle 1985, Sundström et al. 1986, van Grondelle et al. 1987, Bergström et al. 1989, Visscher et al. 1989), is due to general physical considerations about the structure of LHA as well as to the results of fluorescence experiments that are difficult to interpret with only two LHA forms (Freiberg et al., unpublished).

The numerical calculations made in this section suggest that the differences between the kinetics obtained by the fluorescence and the absorption methods may well be due to the inhomogeneity of the domain structure and to the different excitation/recording wavelength (and intensities) used in the measurements. These differences manifest themselves only if τ_1, $\tau_2 \gg \tau_3$, i.e., according to our model, when excitation capture by RC takes place mainly from the longest wavelength LHA forms.

The fast kinetics observed in the absorption experiments is due to the decay directly out of the excited energy level, while the slow kinetics are determined by the general excitation decay within the LHA, which in turn is influenced by the rate of excitation capture by RC. Lowering of temperature results in a decrease in the values of W_1'. In the case (a), this leads to a relative growth of the short-lifetime component in absorption experiments, as the amplitude of the slow component is defined by the W_1'. This is exactly what is seen in the absorption experiments (Sundström et al. 1986, van Grondelle et al. 1987, Bergström et al. 1989, Visscher et al. 1989).

In fluorescence experiments (Borisov et al. 1985, Freiberg et al. 1987, Godik et al. 1988), the fast decay kinetics are less pronounced making it impossible to monitor the directly excited ensemble of pigments. Transfer processes between different spectral pigment forms appear indirectly via the rise times and the decay times of secondary excited pigments. Nevertheless, as shown (case (b)) in fluorescence experiments at low temperature, assuming that $W_1 > W_2 > \tau_3^{-1}$, a clearly nonexponental decay can be observed (see Fig. 3). Both in fluorescence and absorption the decay kinetics of the lowest level (level 3 in Fig. 2) is practically single-exponential, regardless of the relation of the values W_i and W_i'.

4. Moderate excitation intensities. Singlet-triplet annihilation

At high repetition rate, pulsed excitation produces a considerable amount of triplet states. Since the mobility of triplet states is low in comparison with singlet states, the triplet states can compete with photochemically active RC for capture of excitation (Geacintov et al. 1978). It was proposed that the shortening of the fluorescence decay time that occurs with increasing excitation intensity, detected by Freiberg et al. (Freiberg et al. 1989) in the studies of the bac-

teria *Rb. spaeroides* and *Chr. minutissimum*, is due to the singlet-triplet annihilation of excitations in the LHA. An analogous behavior of the decay times was observed in *R. rubrum* (Freiberg et al. 1990a) and it is reproduced in Fig. 1. It can be seen that, at high pulse repetition rate, the average rate of excitation quenching k_C starts to increase gradually at intensities as low as 10^9 photons/cm^2 per pulse (i.e., 10^{17} photons/cm^2 s). It should be recognized that these are not extraordinary intensities and in line with those encountered in sunlight-driven photosynthesis.

Let us assume that the increase of the quenching rate mentioned is due to the singlet-triplet annihilation processes, i.e., the measured excitation quenching rate constant, k_C, is equal to:

$$k_C = k_0 + \gamma_{ST} n_T \,, \tag{2}$$

where k_0 is the rate constant of excitation quenching by closed RC and other modes of monomolecular decay; γ_{ST} is the rate constant of singlet-triplet annihilation.

In typical experiments, samples with thickness of ≈ 1 mm are employed with a characteristic optical density of $A \approx 0.1$ at the excitation wavelength. This suggests that the average concentration of pigments in the sample is about $C \approx 10^{16}$ cm^{-3}. At the excitation intensities of $I \approx 10^{12}$ photons/cm^2 per pulse, the concentration of singlet excitations is of the order of $n_S \approx 10^{12}$ cm^{-3}. Then, at $\tau_\Delta = 12$ ns, formula (1) gives $n_T \approx 10^{14}$ cm^{-3}. So, even at rather low excitation intensities, n_T may become approximately equivalent to C. Consequently, in the calculations of different characteristics as a function of excitation intensity, it is necessary to take into account this saturation effect. With this in mind, Eq. (1) can be rewritten as follows:

$$n_T = \Phi_{IC} n_S (1 - n_T/N_C)\tau_T/\tau_\Delta \,, \tag{3}$$

where N_C is the concentration of the pigment molecules (most probably carotenoids) (Monger and Parson 1977, Rademaker and Hoff 1981, Renger and Wolf 1977, Kingma 1983) which take part in the long-lived triplet state formation.

Using formula (3) and the expression

$$n_S = AI/x \tag{4}$$

to determine the number of the singlet excitations generated by a pulse of fluence I in the sample of thickness x and optical density A, we obtain

$$n_T = \Phi_{IC}\tau_T/\tau_\Delta \frac{AI/x}{1 + \Phi_{IC}\dfrac{\tau_T}{\tau_\Delta}\dfrac{AI}{N_C x}} \,. \tag{5}$$

According to formula (2) k_C is the function of I, and also from the definition of the quantum yield Φ_{IC}

$$\Phi_{IC} = k_{IC}/k_C \,, \tag{6}$$

where k_{IC} is the rate of intersystem crossing, we obtain for the value k_C the following nonlinear relation:

$$k_C = k_0 + \alpha_{ST} \frac{\alpha I k_0/k_C}{1 + \dfrac{\alpha\beta}{C}\dfrac{Ik_0}{k_C}} \,, \tag{7}$$

where

$$\alpha = \Phi^0_{IC} A\tau_T/(\tau_\Delta \cdot x) \,, \quad \Phi^0_{IC} = k_{IC}/k_0 \,, \quad \beta = C/N_C \,. \tag{8}$$

Formula (7) can be rearranged to give:

$$\frac{Ik_0}{k_C(k_C - k_0)} = \frac{1}{\alpha\gamma_{ST}} + \frac{\beta}{C\gamma_{ST}}\frac{Ik_0}{k_C} \,. \tag{9}$$

Based on the experimental data (Freiberg et al. 1990a) included in Fig. 1, Fig. 4 represents the dependencies of the value $Ik_0/(k_C(k_C - k_0))$ on Ik_0/k_C which, in accordance with formula (9), is linear. From the slope of this line the value of $\beta/(C\gamma_{ST})$ can be determined:

$$\frac{\beta}{C\gamma_{ST}} = 3.4 \times 10^{-10} \text{ s} \,. \tag{10}$$

Assuming that $C = 10^{16}$ cm^{-3}, $\gamma_{ST} = 3\beta\ 10^{-7}$ cm^3 s^{-1}. It should be emphasized that when the saturation effects are neglected, i.e., assuming in formula (7) that $\beta = 0$, it is not possible to fit the experimental data. This is directly evident from Fig. 4, since in the case of $\beta = 0$ the graph should be a straight line which is parallel to the x-axis.

The experimental systems under consideration here are suspensions of bacterial chromato-

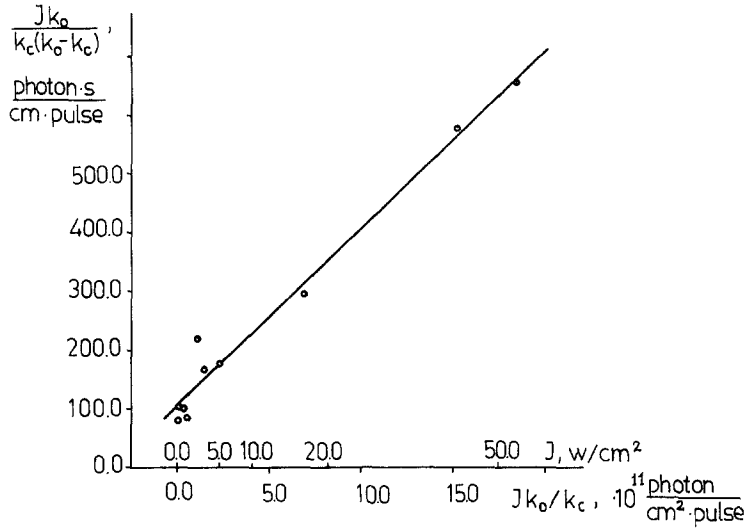

Fig. 4. Dependence of the value of $I/(k_C(k_C - k_0))$ on I/k_C. The points have been calculated from the experimental data presented in Fig. 1. The streight line is approximation of the experimental data.

phores and, in particular, the portion of the membrane in which the bulk of photosynthetic pigments is located. Therefore, it is not quite accurate to assume average concentrations. Estimating the density of pigment molecules in a chromatophore to be on the order of 10^{19} cm^{-3} ($\approx 10^3$ bacteriochlorophyll molecules are contained in a sphere with the radius 200 Å (Junge and Jackson 1982), and also taking into account that the number of carotenoids is about half the number of bacteriochlorophyll molecules, i.e., $\beta = 2$, from formula (10) it is obtained that $\gamma_{ST} \approx 10^{-9}$ cm^3 s^{-1} or, when calculated per single domain, which contains a common LHA and tens of RC (Bakker et al. 1983, den Hollander et al. 1983), $\gamma_{ST} \approx 10^{-5}$ ps^{-1}.

Using the above estimations for γ_{ST} and the fluorescence kinetics data in the region of the intensities of 10^8–10^9 photons/cm^2 per pulse from Fig. 1 (see also Fig. 4), we can infer that $(\alpha\gamma_{ST})^{-1} \approx 100$. Assuming now that the light beam cross-section $S = 0.2$ cm^2, $x = 0.1$ cm, $A = 0.1$ units of optical density, and that the number of domains in the excited region $N_D \approx 10^{11}$, we estimate that:

$$\Phi^0_{IC}\tau_T/\tau_\Delta \approx 500\,. \qquad (11)$$

This result corresponds, within an order of magnitude, to the estimate given in the Introduction on the basis of formula (1).

An interesting question arises: what happens in the samples which lack carotenoids? Monger and Parson (Monger and Parson 1977) have shown that in carotenoidless mutants, at sufficiently high excitation intensities, bacteriochlorophyll triplets are generated. Therefore, the proposed saturation mechanism causing the bleaching of the bacteriochlorophyll's optical transitions from the ground state will work in this case as well. A formula, analogous to relationships drawn in Eqs. (7) and (9) can be obtained by using the corrected β parameter: $\beta = 1 - \varepsilon_T/\varepsilon$, the extinction coefficients of bacteriochlorophyll given in the ground state by ε and in the triplet state by ε_T.

In spite of our qualitative success in explaining the mean fluorescence decay time course at moderate intensities, in the region of $I > 10^{12}$ photons/cm^2 per pulse the calculated curve deviates strongly from the experimental. For example, saturation of carotenoid triplet states may be accompanied with the increase of the LHA bacteriochlorophyll triplet states. If so, a new path for singlet-triplet annihilation emerges, which gives the above-mentioned changes of parameter β. In the region of high fluences ($> 10^{13}$ photons/cm^2 per pulse), it is necessary to allow

for singlet-singlet annihilation. Some higher-order intensity effects, for example triplet-triplet annihilation, which in turn leads to a decrease of n_T, cannot be excluded. Taken together all of these processes conspire to cause the deviation of both the calculated and experimental dependencies of τ_C on I in the region of high intensities (Fig. 1).

The same effects take part in the absorption data. In the experiments by Sundström et al. (1986), van Grondelle at al. (1987), Bergström et al. (1989), Visscher et. (1989) the optical densities of the experimental samples were significant, $A > 0.3$, that leads to the inhomogeneous distribution of the excitation in the sample. This alone is sufficient to course nonexponential kinetics and it could well be one of the reasons for differences between the kinetics measured by the fluorescence and absorption methods.

Triplet generation and singlet-triplet annihilation take place in chloroplasts as well (Breton et al. 1979). Although the original explanation of the dependence of the fluorescence quantum yield on excitation intensity was based on the assumption of the generation of some additional excitation quenchers different from carotenoid triplets, an analysis of the experimental data suggests that saturation effects may be the more imporitant feature. The data can be best fit with the saturation formula (3) if some heterogeneity of the excitation in the sample due to high optical density is taken into account.

5. The region of high intensities. Singlet-singlet annihilation

With the growth of the excitation intensity in the region of 10^{13} photons/cm^2 per pulse and higher, more than one interacting singlet excitation will be created in the domain. This interaction manifests itself in further shortening of the average excitation lifetime (see Fig. 1), the decrease in the fluorescence quantum yield (Paillotin et al. 1979, van Grondelle 1985) as well as the decrease in the probability of excitation capture by RC (Mineev and Razjivin 1987).

The characteristic excitation lifetime, resulting from the singlet-singlet annihilation process, $\tau_{S-S} = (\gamma_{S-S} n_S)^{-1}$, ($\gamma_{S-S}$ is the rate constant of singlet-singlet annihilation), may be defined as a sum of two lifetimes (den Hollander et al. 1983, van Grondelle 1985, Valkunas et al. 1986)[1]:

$$\tau_{S-S} = \tau_m + \tau_s \,. \tag{12}$$

Here τ_m is the time of excitation migration in the domain before interacting with the other excitation, τ_s is the time of the static destruction of the excitation when two excitations occur within the characteristic annihilation radius or when one molecule is doubly excited. For the values τ_m and τ_S the following expressions are obtained:

$$\begin{aligned} \tau_m &= \frac{1}{4} N f_p(N) \tau_{hop} \,, \\ \tau_S &= N \tau_{rel} \,, \end{aligned} \tag{13}$$

there N is the average number of pigment molecules per excitation in LHA, $N = R_D^p/(a^p n_S)$, (R_D is the dimension of the domain, a is the characteristic distance between the 'nearest neighbors' in LHA). τ_{hop} is the characteristic time for excitation hopping to the already excited molecule (inversely proportional to the overlap of the donor emission spectrum with the acceptor absorption spectrum from the excited state, if one uses the language of the conventional Förster theory of dipole-dipole resonance energy transfer (for overview see Agranovich and Galanin (1982))), τ_{rel} is the time of relaxation of the twofold excited state of molecules populated in the annihilation process, $f_p(N)$ is the structure function of LHA (Kudzmauskas et al. 1983, Valkunas et al. 1986) and p is the dimension of the system.

To determine which term in formula (12) controls the annihilation rate in photosynthetic bacteria let us first assume that the migration process is sufficiently fast to satisfy the inequality $\tau_m \ll \tau_S$ which, with regard to expressions (13), takes the form

$$\frac{f_p(N)}{4} \tau_{hop} \ll \tau_{rel} \,, \tag{14}$$

[1] Note that τ_{S-S} is a function of time as n_S changes with time.Therefore, it is correct to define τ_{S-S} as a characteristic lifetime at a certain moment t.

$f_p(N)$ is (in case of two- and three-dimensional systems) a weak function of N. Its value increases with the increasing N and, in the limit of large N, becomes equal to 0.5 (at $p=3$) or $(2\pi)^{-1}$. ln(1.85 N) (at $p=2$) (Kudzmauskas et al. 1983). Hence, inequality (14) will be satisfied if the condition

$$0.1\tau_{hop} \ll \tau_{rel} \tag{15}$$

holds.

It is well known (Agranowich and Galanin 1982) that in case of incoherent excitation transfer

$$\tau_{hop} \gg T_2, T_2',$$

where T_2 and T_2' are the excitation phase relaxation times in the first and the twofold excited singlet states, respectively. T_2 and T_2' can be determined from the reverse width of the corresponding homogeneously broadened absorption bands. For chlorophylls at room temperatures (when the spectra are predominantly homogeneously broadened) an order of magnitude estimation gives $T_2 \approx 10^{-13}$ s (Kenkre and Knox 1976). As can be inferred from the absorption difference spectra (Borisov et al. 1982, Nuijs et al. 1985), for bacterial photosynthesis, T_2' is a value of the same order. Consequently, in case of incoherent excitation transfer, τ_{hop} must be of the order of picoseconds and, proceeding from formula (15), τ_{rel} cannot be less than several picoseconds either.

The expression for τ_{rel} (Valkunas et al. 1986) is of the following form

$$\tau_{rel} = \gamma^{-1} W_2 / W_1 , \tag{16}$$

where γ is the intramolecular rate of excitation relaxation from the doubly excited state to a lower-lying, relatively long-lived intermediate state. W_2 is the rate of the decay of the doubly excited molecule into two single-excited molecules and W_1 determines the rate of the reverse process. An analysis of the difference spectra of bacterial chromatophores (Kudzmauskas et al. 1988) suggests that γ^{-1} cannot be more than a picosecond and according to inequality (15) $\tau_{hop} < 1$ ps. In the limiting case, when $W_2 \to 0$ τ_{rel} becomes controlled by the value W_1 (Valkunas et al. 1986) and inequality (15) could be satisfied only when the value $1/W_1$ is of the order of 10 ps. Such an assumption extends considerably the ranges of applicability of the theory of Pailotin et al. (1979). Note that this theory is also applicable in the case of coherent excitons when the reverse inequality (15) is satisfied. However, from the difference spectra (Borisov et al. 1982, Nuijs et al. 1985), it is explicitly evident that the absorption spectra of chlorophylls from ground and excited states are close to one another as are the corresponding overlap integrals for excitation transfer rate between two excited molecules, W_1, and between the excited and nonexcited molecule, W.

It follows from what was said above that in case of incoherent excitation migration, the reverse assumption with respect to (14) may appear to be more natural. This means that the process of singlet-singlet annihilation is probably migration-controlled, $\tau_{S-S} \approx \tau_m$, and the correct theory of excitation annihilation should take account of correlation effects between the excitations.

This view also suggests an alternative approach to that proposed by Pearlstein (Pearlstein 1982) to investigate whether migration or trapping by the RC controls the excitation quenching rate in LHA at low pumping intensities. On the qualitative level, the highly efficient migration-controlled singlet-singlet annihilation processes, which manifest themselves after only a few excitations are in the domain, assumes a rather fast excitation migration when excitation diffusion radius at least equal to the domain size. Thus, one might expect that at low excitation concentration the quenching is trapping not migration limited. This inference is supported by the following numerical analyses. The time of singlet-singlet annihilation τ_{S-S} in case of two excitations in the domain *R. rubrum* was estimated to be equal to 400 ps (Bakker et al. 1983). Assuming a migration limited annihilation in two-dimensional LHA and taking $N_D \approx 1.5 \times 10^3$, one gets from formula (13) an estimate of τ_{hop} to be of the order of 1 ps. As $\tau_{hop} \simeq W^{-1}$ (see before), it follows that the time of migration along the photosynthetic unit containing some tens of pigment molecules should take no more

than 5 ps. Since the average lifetime in LHA is at least 60 ps, the excitation decay is most probably capture-limited.

As to the conclusion drawn by several authors (see Introduction) from absorption measurements that at low pumping intensities the excitation lifetime is determined by migration, it need not be so. The reasons are the relatively high pumping intensities and high optical densities of the samples used in these experiments (see discussion in Section 4). For example, the modeling of the experiment of Danielius and Razjivin (1988) by using a lattice type photosynthetic unit (Kudzmauskas et al. 1983, Valkunas et al. 1986) shows that within the experimental errors the results are explainable by both models in which quenching is capture-limited as well as models in which it is migration-limited.

6. Spectral changes at high excitation intensities

Rather peculiar difference-absorption spectra at high excitation intensities have been reported (Borisov et al. 1982, 1984, Nuijs et al. 1985) which lead to the notion of the so-called minor and major LHA forms. It was shown by Kudzmauskas et al. (1985, 1986) that these spectral changes can be interpreted, taking into account the population of higher excited states of LHA pigments via singlet-singlet annihilation. Actually, the difference spectrum at low excitation intensities is determined by the difference of the absorption cross sections $\sigma_1(\lambda) - \sigma_0(\lambda)$, where $\sigma_1(\lambda)$ describes the absorption from the excited state of the system and $\sigma_0(\lambda)$ determines the absorption cross-section from the ground state of the chlorophyll molecules (λ is the wavelength). The amplitude of the response is determined by the number of excitations in the domain n_S. At high excitation intensities the singlet-singlet annihilation becomes very efficient and, due to the finiteness of the life-time of higher excited states, their population becomes significant during the excitation pulse. The difference spectrum in such a case is determined by the above-mentioned difference $\sigma_1(\lambda) - \sigma_0(\lambda)$ as well as by the difference $\sigma_H(\lambda) - \sigma_0(\lambda)$, where $\sigma_H(\lambda)$ s the absorption cross-section from the higher excited states. The relative influence of both terms is determined by the ratio of the population numbers of first excited state n_S and higher excited state n_H. This is the cause of the distinct changes in difference spectra at high excitation intensities being related to the appearance of the so-called major LHA spectral form. At low intensities the appearance of the minor LHA spectral form is determined by the difference of $\sigma_1(\lambda) - \sigma_0(\lambda)$. The value $\sigma_1(\lambda)$ can be also influenced by higher order excitated correlation effects which may be manifested through the displacement of the spectrum towards either the long or the short-wavelength region with increases in the excitation intensity (Agranovich and Efremov 1980). Such displacement is induced by changes of the electronic terms of the excited molecule due to the presence of another excited molecule in the vicinity. With bacterial chromatophores, a short wavelength displacement of the fluorescence spectrum with the increasing intensity is really observed (Vos et al. 1986, Freiberg et al. 1989). It cannot be excluded that an analogous dynamical interaction induces also the peculiar difference absorption spectra reported by Nuijs et al. (1985) at not very high excitation intensities.

The displacement of fluorescence spectra, however, may also be due to other reasons. In particular, local heating of separate domains due to a large number of simultaneous annihilation events at high excitation intensities may be an important factor. Let us estimate the possible heating effect. The maximal amount of the energy liberated and dissipated by the vibrational subsystem due to annihilation is $h\nu n_S$ ($h\nu$ is the energy of the quantum of exciting light, n_S is the number of singlet excitations in the domain). Assuming for simplicity that a separate domain can be presented as an isolated system, the upper limit of the local temperature of the domain may be estimated by the following formula:

$$V_D C_V T = h\nu n_S \tag{17}$$

where V_D is the volume of the domain, C_V is its heat capacity and T, temperature. If it is assumed that the diameter of the domain heated is $L = 400$ Å and its heat conductance is of the order of $\kappa = 0.1$–1 cm/s, then the characteristic time of equalization of the domain temperature with that of the thermostat is $L^2/\kappa \approx 10^{-10}$–$10^{-11}$ s.

In the region of low temperatures

$$C_V = \alpha T^3 . \quad (18)$$

Introducing the values $V_D \approx 6 \times 10^{-17}$ cm^3 and $\alpha \sim 100$ erg/K^4 cm^3, we obtain that $T = 7$ K and 12 K, respectively for 6 and 60 excitations in the domain. So, in this case the local heating effect is small and the question whether the described heating mechanism determines the short-wave spectral displacement observed (Vos et al. 1986), at low temperatures, remains open.

At room temperatures and in the case of excitation with high-intensity single pulses, no spectral displacement was observed (Vos et al. 1986). This allows us to estimate C_V in the high-temperature limit. Taking $n_S = 60$, $T < 300$ K and $C_V = \text{const.}$, we have

$$C_V > 5 \times 10^{19} \text{ cm}^{-3} . \quad (19)$$

On a high-frequency excitation of the system high concentrations of triplet excitations are generated exceeding that of singlet excitations by several orders of magnitude. In this case and at sufficiently high intensities the local heating may result mainly from triplet-triplet annihilation processes (if $n_S = 60$, $n_T \ll 6 \times 10^3$). This means that in the domain almost every pigment is in the triplet excited state. The upper limit of the local heating in this case may be estimated by the formula analogous to formula (17):

$$V_D C_V T < E_T n_T , \quad (20)$$

where E_T is the energy of triplet excitation. Using estimate (19) for C_V, we obtain

$$T < 3 \times 10^3 \text{ K} . \quad (21)$$

Consequently, in principle, the local heating mechanism described may determine the spectral shift of the emission bands observed (Freiberg et al. 1989) at room temperatures in case of high pumping intensities.

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