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Kinetics of picosecond bacteriochlorophyll luminescence in vivo as a function of the reaction center state

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Bacteriochlorophyll (BChl) luminescence lifetimes (τ) were measured in purple bacteria *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* at low-excitation pulse energy with the use of a picosecond luminescence spectrochronograph of high sensitivity and high time-resolution. Average high-frequency excitation light density was changed from about $1 \cdot 10^{13}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} up to $1 \cdot 10^{17}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1}. Maximal energy density in a single pulse was in the range 10^{-14}–10^{-10} J/cm^2, which completely rules out nonlinear exciton interactions. In this range τ increased as a function of excitation light density from about 60 ps to 210 ps. Luminescence yield (ϕ) for the bacteria investigated measured under continuous or picosecond excitation changed in a similar manner as τ. The luminescence increase was shown to accompany the conversion of the reaction centers to the closed, photooxidized state. Luminescence decay of *R. rubrum* and *Rps. sphaeroides* chromatophores without any chemical additions was well approximated by a single exponential component both at low and at saturating intensities of exciting light. The time necessary for the primary charge separation to occur was shown to be 60 ± 10 ps. The pairwise jump-time of excitation-energy transfer, as well as excitation-diffusion characteristics were estimated from these data. On the basis of life-time measurements in the state of active photosynthesis, the quantum yield of the primary charge separation in the reaction centers was estimated to be equal to 0.95 ± 0.02. In intact cells as well as in chromatophores in the presence of reducing agents, a nanosecond component of emission decay was also observed. The relative amplitude of this component, being several percent of the picosecond one at low-excitation intensity levels, increased (2–3)-times with excitation density. Its life-time was estimated to be 3 ± 1 ns. The nanosecond component appeared only under conditions when a part of the reaction centers were converted to the closed state PQ^-.

Introduction

There is a convincing body of evidence that the two main functions that chlorophyll (Chl) or bacteriochlorophyll (BChl) performs during photosynthesis, viz., light absorption and excitation energy transductions, are performed by two different types of Chl protein: light quanta are absorbed and collected by the so-called antenna or light-harvesting Chl, while excitation energy is transformed into an electrochemical form with participation of chlorophyllous pigments of the reac-

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Abbreviations: Chl, chlorophyll; BChl, bacteriochlorophyll; TMPD, tetramethyl-*p*-phenylenediamine.

tion centers [1]. It was first established by Duysens [2,3] that at least part of the excitation energy comes to the reaction centres via singlet excited states of antenna Chl. These data were supported by observations from many laboratories and it is now widely accepted that Chl singlet-excited states are the main carriers of excitation energy to the reaction centers and the mechanism of the transfer is that of resonance-energy migration (for reviews, see Refs. 1 and 4–7). The main approach used to elucidate the above problems was time-resolved luminescence spectroscopy. This approach is based on the fact that excitation energy diffuses very quickly to the reaction centers via Chl singlet-excited levels and that the quantum yield of charge separation is high. As a result, spontaneous Chl luminescence decay should reflect the kinetics of the sequence of events of energy transfer and trapping by reaction centers as well as the subsequent events of excitation stabilization. The first observations of picosecond lifetimes of antenna Chls are those of Refs. 8–10, in which phase fluorometry was utilized. However, the time-lever method, elaborated in these works (see also Ref. 11), enabled a reliable estimation of only the upper limit of BChl life-time under low-light photosynthesis conditions and values of $\tau \lesssim 40$–80 ps were obtained. As for the subsequent work [12,13], the employment of picosecond light pulses of high intensity could hardly exclude the occurrence of multiple exciton-exciton interactions which may distort the fluorescence kinetics observed (see, for example, Ref. 14). Thus, at the present time, a divergence exists between the values of luminescence lifetimes of purple bacteria at low-light conditions measured by different investigators from 10–80 ps [8–11,13,15] to 250–400 ps [12,16]. In addition, in a number of works nanosecond components were also observed [8–10,12,15]; however, their nature was not clearly elucidated.

The main task of this work is a systematic investigation at ambient temperature of luminescence decay kinetics of purple bacteria, the simplest photosynthetic systems by using a novel experimental device, a picosecond spectrochronograph, whose sensitivity permitted the measurement of picosecond lifetimes under conditions of low-light level photosynthesis. The state of the reaction centers was checked by independent measurements under continuous excitation of equal excitation density.

Materials and Methods

Cells of *Rhodospirillum rubrum* (wild type No. 1. MGU), and *Rhodopseudomonas sphaeroides* (wild type No. 1. MGU) were grown as described in Ref. 17, and chromatophores were isolated as in Ref. 18. Chromatophores were stored at 0°C under anaerobic conditions in 50 mM Tris-HCl (pH 7.5) and diluted by the buffer to a final BChl concentration $(3–6)\cdot 10^{16}$ molecules per ml just before measurements. Bacterial cells were used in their own cultural medium 3–5 days after inoculation from a previous culture.

The apparatus for picosecond luminescence life-time measurements was described earlier [19–21] in detail. Briefly, a mode-locked CW oxazine 1 or oxazine 750 dye laser (tuning ranges, 685–820 nm and 345–410 nm, with a frequency-doubling; pulse duration at the half-height level, 3 ps; average power, up to 200 mW), synchronously pumped at 82 MHz by a krypton-ion laser, was used as a source for excitation. Emission, viewed from the same cell wall where exciting light falls (which excludes the effects of reabsorption), came through a double-grating monochromator with a bandwidth of about 4 nm (in some cases through narrow-band filters) and was registered by a synchroscan streak camera, working in synchronism with the dye laser operation. In order to avoid possible damage effects of the exciting light, a flow cell with a flow rate of 60 mm/s was employed. The average exciting-light density was attenuated with the aid of gray filters and checked by a radiometer, or a calibrated photoelement. The same detector was used to monitor the continuous-light density in parallel measurements of the relative quantum yield of luminescence and the fraction of photooxidized reaction centers P^+/P_0; the latter was determined as a normalized absorption change at about 800 nm (and measured with a double-beam differential spectrophotometer). Luminescence yield was measured with an Aminco-Bowman spectrofluorimeter. Blue excitation at about 400 nm was used as actinic light in this case. All

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decay curves were deconvoluted as a sum of two exponential components, utilizing the apparatus response function which was stored independently.

Results

Luminescence life-time of *R. rubrum* chromatophores without any chemical addition is shown in Fig. 1 as a function of exciting light density in the range from $3 \cdot 10^{13}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} to $1 \cdot 10^{16}$ photons $\cdot$ cm$^2 \cdot$ s^{-1}. For this particular sample, at lower intensities signal-to-noise ratio was too low. The corresponding dependences of ϕ and P^+/P_0, also shown in Fig. 1, indicate that in the range of light densities employed, a conversion of the reaction centers into photooxidized state occurs. In response to this process, ϕ (excited by continuous as well as by high-frequency pulse excitation) and τ increase in a quite similar manner. Experimental decay curves at each of the intensities used are well approximated by a single exponential component (Fig. 1, see also Refs. 21 and 22).

Chromatophores without additions have, as a rule, their light-intensity curves rather prolonged and shifted to lower intensities, as compared with those for intact cells. We used additions of TMPD together with sodium ascorbate or mercaptoethanol in order to improve light-intensity dependences of P^+/P_0, τ and ϕ and to shift them to higher intensities [21,22]. As an example, light-intensity dependences of luminescence yield of *Rps. sphaeroides* chromatophores without additions and in the presence of $5 \cdot 10^{-5}$ M TMPD together with $1 \cdot 10^{-3}$ M mercaptoethanol are shown in Fig. 2. It can be seen that in the latter case this dependence is considerably improved. However, fast accumulation of the reaction centers in the state PQ^- has been shown to occur under these conditions (P is the reaction center BChl special pair, Q is the primary quinone acceptor). This is revealed by diminishing (in anaerobic conditions by complete disappearance) of the steady-state differential absorption changes, reflecting photooxidation of the reaction centers (see, e.g., Ref. 23) and by large increase in the luminescence lifetime, as measured by the phase-shift method [24]. In our measurements luminescence decay of chromatophores in the presence of TMPD and reducing agents was found to be obviously two-exponential at suffi-

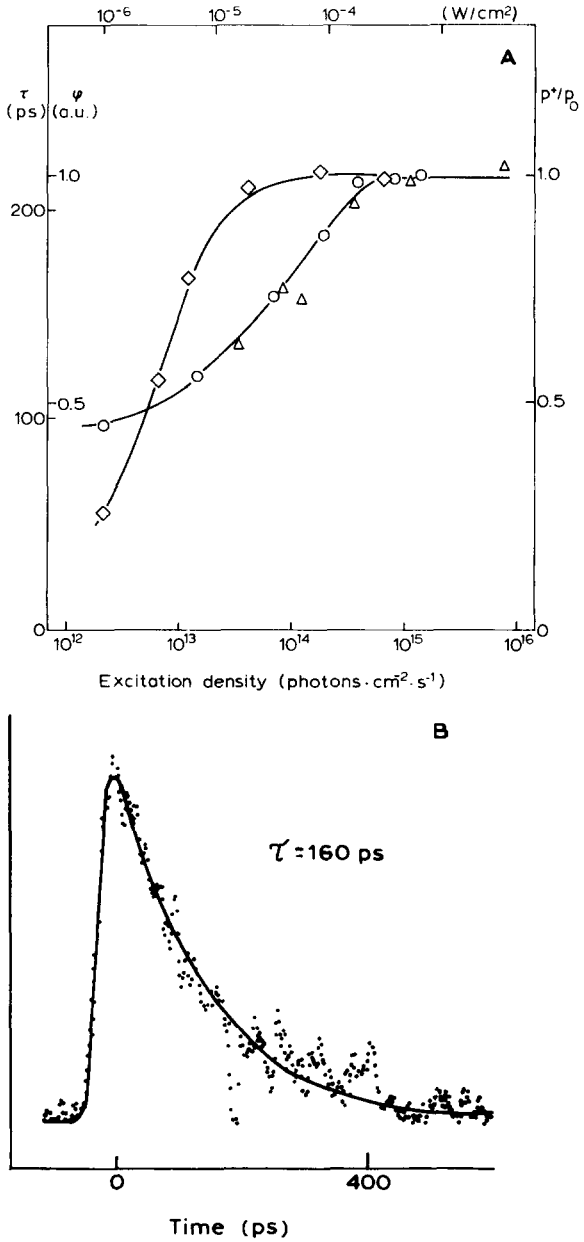

Fig. 1. (A) Exciting-light density dependences of pure *R. rubrum* chromatophores emission lifetime τ (△———△), excitation at 385 nm, detection at 920 nm; relative luminescence yield ϕ (○———○), excitation at 404 nm, continuous light, detection at 920 nm. (◇———◇), the normalized fraction of photooxidized reaction centers P^+/P_0. (B) shows the kinetics of luminescence decay of *R. rubrum* chromatophores without additions for exciting light density of approx. $1 \cdot 10^{14}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} (As the emission lifetime is independent of emission wavelength within the whole near-infrared emission band, the detection bandwidth used is specified neither in this nor in the following figures.)

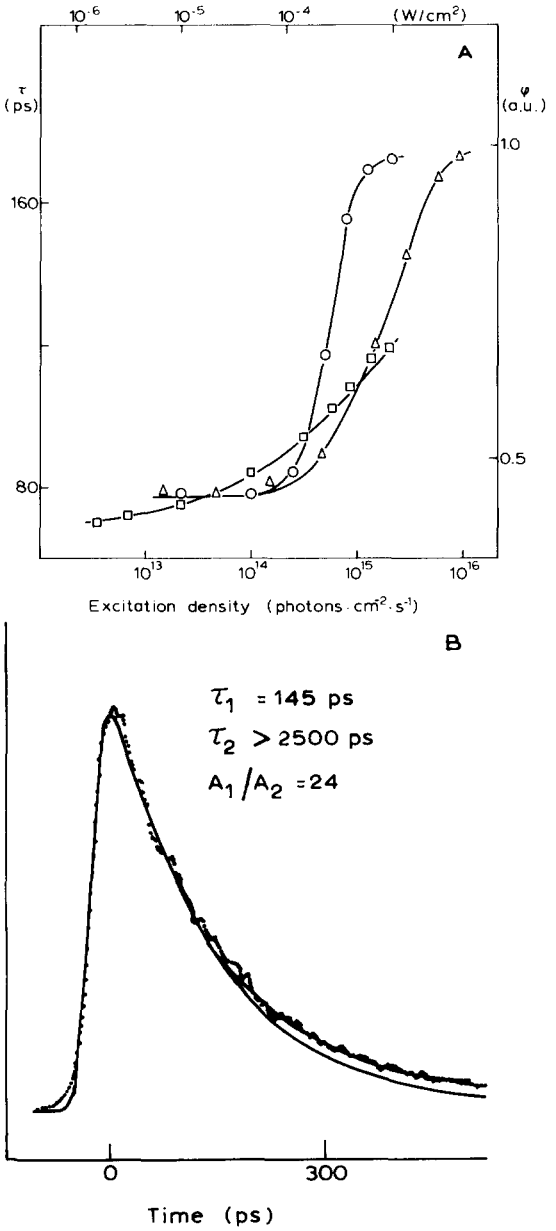

Fig. 2. (A) The lifetime τ of the picosecond component of the emission (△———△) and the relative emission yield ϕ (○———○) of *Rps. sphaeroides* chromatophores in the presence of $5 \cdot 10^{-5}$ M TMPD and $1 \cdot 10^{-3}$ M mercaptoethanol (the wavelength of excitation and registration as in Fig. 1) as a function of excitation light density, and the same for the emission yield of chromatophores without additions (□———□). (B) The kinetics of luminescence decay of *Rps. sphaeroides* chromatophores in the presence of $5 \cdot 10^{-5}$ M TMPD and $1 \cdot 10^{-3}$ M mercaptoethanol; excitation density of $3 \cdot 10^{15}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} was employed. The unbroken curve shows a single exponential deconvolution of the experimental curve.

ciently high exciting light, both in aerobic and anaerobic conditions. Usually, when exciting light density exceeded $5 \cdot 10^{14}$–$1 \cdot 10^{15}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1}, which was large enough to trap part of the reaction centers in the state PQ^- during several seconds of exposure to exciting light, a nanosecond component appeared together with the picosecond one. The kinetics of the luminescence decay of *Rps. sphaeroides* chromatophores under aerobic conditions for exciting light density of $3 \cdot 10^{15}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} is shown in Fig. 2. It can be seen that the decay is well simulated by two components, the relative amplitudes of which differed approximately by a factor of 50 at low-excitation densities and by a factor of 25 at saturating-light densities. The light-intensity curve for the picosecond component is shifted to higher intensity as compared with chromatophores without additions (compare Figs. 1 and 2) so that in this case low intensity level is achieved reliably. The life-time of the picosecond component behaves as a function of the light density more or less in the same manner as in chromatophores of *R. rubrum* without additions. The life-time of the nanosecond component does not depend noticeably on the excitation intensity, and remains within a range of 2–4 ns, but, as mentioned above, its relative amplitude increases with increasing light density.

Luminescence decay of intact cells of *Rps. sphaeroides* was found to be also two-exponential both at low and saturating light (Fig. 3). Corresponding light-intensity dependences of P^+/P_0, τ and ϕ show (Fig. 4) that for this object low-exciting light conditions are achieved at about $2 \cdot 10^{14}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1}. The relative amplitude of the nanosecond component as a function of exciting light density is also shown in Fig. 4. The presence of the nanosecond component at low intensity is most likely caused by the presence of the reaction centers with the primary acceptor chemically reduced by endogenous donors. A substantial increase in the relative amplitude of the nanosecond component was observed at approximately $1 \cdot 10^{15}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1}; under these conditions this component appeared also in the emission of *R. rubrum* and *Rps. sphaeroides* chromatophores (Fig. 2). Photochemical trapping of the reaction centers in the state PQ^- sets in at this intensity, when the

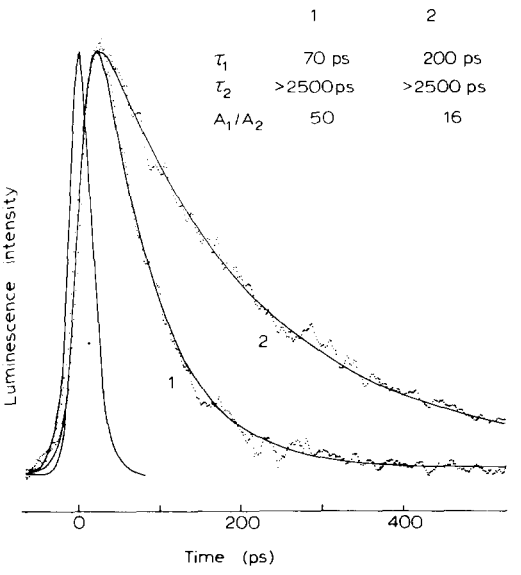

Fig. 3. The kinetics of luminescence decay of intact cells of *Rps. sphaeroides* for two extreme exciting-light densities (see Fig. 4): (1) approx. 10^{14} photons·cm^{-2}·s^{-1} and (2) approx. 10^{16} photons·cm^{-2}·s^{-1}. Excitation and detection wavelengths are 385 nm and 920 nm, respectively. Excitation pulse profile is also shown.

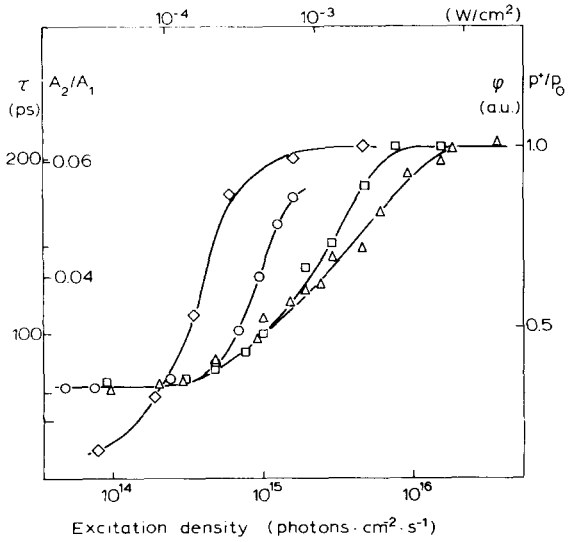

Fig. 4. Exciting-light density dependences of (1) the life-time of the picosecond component of the emission of *Rps. sphaeroides* cells (△——△); (2) the relative amplitude ratio, A_2/A_1 of the nanosecond and picosecond components of the emission (□——□); (3) the relative luminescence yield ϕ (○——○); and (4) the normalized portion of photooxidized reaction centers P^+/P_0 (◇——◇). In all cases, the conditions of excitation and detection are the same as in Fig. 1.

appropriate conditions are maintained. As a result of non-exponential decay, the quantum yield of luminescence, excited by continuous light, should increase (and, as shown in Figs. 2 and 4, it does) more sharply than the life-time of the picosecond component. This latter value was found to be 66 ± 10 ps at low and 210 ± 10 ps at saturating light, which correlates well with the data on *Rps. sphaeroides* chromatophores and with our previous measurements of τ light-intensity dependences in *R. rubrum* chromatophores [21,22]. These data are in good qualitative agreement with previous indirect phase-fluorimetry data [9,15,25].

When all of the reaction centers in *R. rubrum* chromatophores were chemically reduced by 10^{-3} M sodium dithionite, the nanosecond component had maximal amplitude and the life-time of the picosecond component did not depend on exciting light density, being 20–30% longer than that in untreated chromatophores at low exciting-light conditions (as in Ref. 21). The same behavior was observed for *R. rubrum* chromatophores in the presence of $5 \cdot 10^{-5}$ M TMPD and $5 \cdot 10^{-3}$ M sodium ascorbate under anaerobic conditions.

In contrast to plant systems (see, for example, Ref. 20), no noticeable τ dependence on emission wavelength was observed either for *R. rubrum* or for *Rps. sphaeroides*. The dependences of τ on excitation wavelength, studied for *R. rubrum* chromatophores, show that at low-light conditions there are no noticeable life-time changes upon excitation at 800 nm (where about half of the excitation quanta are absorbed by P-800 of the reaction centers) and at 375 nm, which ensures the conditions corresponding closely to those of uniform excitation.

Discussion

Excitation-energy transfer and trapping in purple bacteria

It follows from the data of this work, as well as from our previous publications [21,22] that the luminescence life-time of purple bacteria approximates 50–70 ps at low (less than about $1 \cdot 10^{14}$ photons · cm^{-2} · s^{-1}) average exciting high-frequency light density. This value is somewhat lower in the most intact object, bacterial cells, as compared to the value in chromatophores. The

reverse is obtained for τ values at saturating light (Figs. 2 and 4). Apparently, the treatments used for the preparation of chromatophores from cell membranes result in irreversible damage of some part of the reaction centers. The low-light values, obtained for a luminescence life-time of τ_{min}, agree well with earlier data, estimated by the phase-shift method [8–10,15] and with the data of Ref. 13, where the value of 100 ± 50 ps was measured for *Rps. sphaeroides* 8.4.1, but are several times shorter than those determined in a number of previous works [12,16]. This difference may be due to either different conditions of measurements or different objects employed.

The correlation observed between τ, ϕ and the state of the reaction centers (Figs. 1 and 4) confirms the singlet-singlet character of excitation migration in bacterial photosynthesis and a multicentral, or lake-model, organization of photosynthetic units elaborated in Refs. 1 and 2. The last conclusion is drawn from the difference in light-density dependences of ϕ and P^+/P_0. In case of isolated photosynthetic units the direct proportionality between ϕ and P^+/P_0 is expected (see Refs. 3, 7 and 26 and references therein).

Thus, some 50–70 ps after light absorption, the photoinduced excitation terminates its diffusion within antenna BChl at a reaction center, where it is trapped and, at least partly, stabilized. The probability of returning of the photomobilized electron from the level, where it finds itself 60 ps after the creation of photoinduced excitation, to that of P*-870, is not high under conditions of active photosynthesis, since luminescence decay in the picosecond time range was well approximated by a single exponential component: the nanosecond emission is observed only under conditions when a part of the reaction centers was in the state PQ^-. (However, some early steps of excitation-energy trapping and stabilization may be reversible on the picosecond time-scale and may reveal themselves in the luminescence decay observed [28,29].) Proportional changes of the integral emission yield of *R. rubrum* chromatophores without additions at continuous light excitation, as well as of τ and ϕ, measured as the area under the decay curves, corroborate this conclusion.

The values of τmin of the order of 50–70 ps, obtained with *R. rubrum* and *Rps. sphaeroides* chromatophores, as well as with *Rps. sphaeroides* cells, is a direct manifestation of the very high values of the quantum yield of primary charge separation in the reaction centers (ϕ_{ph}) (see Appendix for details):

$$\phi_{ph\,max} = \frac{K_{ph}}{K_{ph} + K_{\Sigma}} = \frac{\tau_{ant} - \tau_{min}}{\tau_{ant}}$$

where K_{ph} is the macroscopic rate constant of excitation energy migration and trapping, $K_{\Sigma} = 1/\tau_{ant}$ is the rate constant of trivial energy losses by fluorescence and non-radiative quenching in antenna BChl. The life-time measurements in isolated pigment-protein complexes of *R. rubrum* devoid of the reaction centers [25] or in reaction center-less mutant of *Rps. sphaeroides* PM-8 [13] show that $\tau_{ant} \simeq 1000$–1500 ps. This gives for the state of active photosynthesis (nearly all the reaction centers are in open state): $\phi_{ph\,max} = 0.95 \pm 0.02$. This value agrees well with the luminescence yield and life-time increases of 3–3.5-times under transition from active to saturated photosynthesis (Figs. 2 and 4) when the quenching properties of closed reaction centers are taken into account [25]. As was shown earlier [25,26] and as follows from the data obtained, closed reaction centers are more efficient quenchers of excitations than antenna BChl molecules (compare our values of τ for saturating light, $\tau_{max} \simeq 200$ ps, and $\tau_{ant} = 1000$–1500 ps, obtained for isolated antenna complexes [25]). It means that for a lake-type of photosynthetic unit, the quantum yield of excitation trapping by open reaction centers at intermediate intensities should be less than $\phi_{ph\,max}$, being equal to $\phi_{ph} = (\tau_{max} - \tau)/\tau_{max}$.

Supposing the case of homogeneous and isotropic migration, the pairwise jump-time of excitation energy transfer τ_j can be estimated. In case of isotropic migration in two-dimensional square lattices and the absolute trap [5], the following relation is valid:

$$\tau_{min} = (0.32N \ln N + 0.20N)\tau_j$$

where N is the number of antenna BChl molecules per reaction center and which equals 30–35 for *R. rubrum* and 140–160 for *Rps. sphaeroides*. Hence, τ_j for *R. rubrum* is estimated to be about 1 ps. For

Rps. sphaeroides τmin is approximately equal to that in *R. rubrum,* although the number of antenna molecules per reaction center is about 5-times larger. It means that excitations from B800 and B850 forms of antenna quickly (less than in several picoseconds) reach the long-wavelength one, where excitation trapping proceeds. A similar conclusion was achieved in Ref. 29 on the basis of fluorescence yield studies. This justifies the rate-limiting step being either excitation-energy transfer from the long-wavelength antenna BChl form to a reaction center, or excitation trapping and subsequent stabilization by reaction centers. This point will be further elucidated in a subsequent publication. The values of the jump-times obtained correspond to intermolecular distances of about 2 nm, according to Förster's formula:

$$\tau_J = \tau_0 \left(\frac{R}{R_0} \right)^6 q^{-1}$$

where τ_0, the intrinsic lifetime, is equal to 18 ns [1], R_0 is the Förster's radius equal to 8 nm [13], q is the number of nearest neighbours ($1 \leqslant q \leqslant 4$).

A phenomenological isotropic excitation diffusion constant, D, can be calculated from these data. In the case of random walk in simple quadratic lattice $D = R^2/4\tau_J$ [30], which gives $D \gtrsim 1 \cdot 10^{-2}\ \mathrm{cm^2 \cdot s^{-1}}$. The corresponding excitation diffusion length, L, during excitation life-time satisfies the relationship $L \simeq (D\tau)^{1/2}$ and for low-light conditions L is longer than only about 7 nm. All the above estimations are made for the case of diffusion-limited migration. The value of L thus obtained is evidently too small to ensure the multicentral organization of photosynthetic unit, which is suggested by our quantum yield and life-time light-intensity dependence measurements. In the opposite case of trap-limited migration [21,22] the values of D and L will be much larger. The value of $D > 10^{-3}\ \mathrm{cm^2 \cdot s^{-1}}$ was also found in green plant chloroplasts [40]. Thus exciton diffusion coefficients for bacteriochlorophyll and chlorophyll in vivo appear to be close to the values reported for typical organic crystals [31]. However, they are 4 or 5 orders of magnitude less than D for large-radius excitons in semiconductors.

The rate of excitation transfer obtained is comparable or even higher than vibrational relaxation rates of matrix-isolated organic molecules in the lowest excited electronic states [32]. This means that electronic excitation transfer from nonrelaxed vibrational state (hot transfer) is probable. The theory of such a transfer is first given in Refs. 33 and 34, but whether it is realized between Chl molecules in vivo is still obscure. The relevant systems should have a good overlap of the donor hot luminescence spectrum with the absorption spectrum of the acceptor and the characteristic dependence of the transfer probability on the exciting light frequency [35].

Nanosecond components of the emission and the values of absolute luminescence yield of purple bacteria

It is shown that the decay of luminescence of purple bacteria investigated contains at least two components. Intact cells of *Rps. sphaeroides* reveal the nanosecond component even at low exciting light conditions. This finding explains why nonproportional (and even sometimes antibatic) behavior was observed earlier in luminescence yield and lifetime, measured by the phase-shift method [8–10,15]. The nanosecond component appears only when there is a possibility of finding a fraction of the reaction centers in the state PQ^-. This kind of emission, kinetically studied in detail earlier [27], represents a radiative decay of charges separated in the reaction centers with the primary acceptor in a reduced state.

Taking into account the contribution from these nanosecond emissions, the values of the absolute luminescence yield may be obtained from our data with the use of the relation $\phi = \tau/\tau_0$. The values of ϕ, thus obtained for *Rps. sphaeroides* from the data of Fig. 4, are approx. 1% at low and approx. 3% at saturating exciting light, which is about 2-times lower than those measured directly [36]. The same is true for *R. rubrum* chromatophores. The possibility cannot be excluded that component(s) with relatively long life-times contribute to the overall emission yield, their relative amplitudes being less than about 1%. At present, our instrument cannot resolve such low-intensity emissions due to its dynamic range limitations.

Finally, some remarks should be made on recent data on energy transfer and trapping in purple bacteria obtained with the use of the picosec-

ond absorption technique [37,38] which are in contradiction with our and other fluorescence studies. In the above-cited works the time of excitation decay was found to be almost independent of the reaction center state (open or closed). We believe that the discrepancy is due to different modes of light excitation (see also Ref. 21). In fact, the minimal excitation used in [37,38] was $3 \cdot 10^{14}$ quanta per cm^2 in a single picosecond pulse at the wavelength of 920 nm. It corresponds approximately to one excitation per 130–140 antenna molecules in *R. rubrum*. Contrary to this, in the present work the excitation density in a pulse was more than (10^7–10^8)-times lower. It should be born in mind that in the case of trap-limited migration and a lake-model organization of photosynthetic units, the criterion of one quantum per reaction center usually employed may not be adequate to ensure the absence of excitation-excitation interaction and annihilation and, thereby, the conditions of low-light photosynthesis level [24]. In the absorption-mode studies [37,38], the quantum yield of photosynthesis was found to be higher than 0.6. Taking into account that in this work $\phi_{ph\,max} = 0.95 \pm 0.02$, it may be concluded that in the works [37,38] up to 30–40% of excitations were lost in some nonlinear processes, which could obscure to some extent the kinetics observed. The absence of fluorescence yield increase in the range of 10^{13}–10^{16} photons per cm^2 per pulse for a number of purple bacteria studied in Ref. 13 indicates that the rate of excitation trapping by the reaction centers does not greatly exceed that of singlet-singlet annihilation. In contrast to the case of purple bacteria, a fluorescence yield increase was observed in spinach chloroplasts in response to the transition of the reaction centers to the closed state, showing that in plants the rate of excitation capture by open reaction centers is much higher than that of singlet-singlet annihilation [39].

Appendix

By definition:

$$\phi_{ph} = \frac{K_{ph} \cdot P/P_0}{K_{ph} \cdot P/P_0 + K_\Sigma}$$

and:

$$\phi_{ph\,max} = \frac{K_{ph}}{K_{ph} + K_\Sigma}$$

where K_Σ is the first-order rate constant of trivial energy losses in antenna BChl by fluorescence and internal conversion; K_{ph} is a macroscopic rate constant of excitation energy migration and trapping by open reaction centers; P/P_0 is the relative fraction of open reaction centers. On the other hand, the fluorescence lifetime is equal to:

$$\tau = \frac{1}{K_\Sigma + K_{ph} \cdot P/P_0} \qquad \text{(A-1)}$$

and under conditions of low-light photosynthesis level:

$$\tau\,\mathrm{min} = \frac{1}{K_{ph} + K_\Sigma} \qquad \text{(A-2)}$$

The rate of trivial energy losses may be obtained from the kinetics of fluorescence decay of the samples, free of the reaction centers:

$$\tau_{ant} = \frac{1}{K_\Sigma} \qquad \text{(A-3)}$$

It can easily be obtained from Eqns. A-2 and A-3 that:

$$\phi_{ph\,max} = \frac{\tau_{ant} - \tau_{min}}{\tau_{ant}}$$

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