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Excitation transfer in chlorosomes of green photosynthetic bacteria

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The formation of excited states and energy transfer in chlorosomes of the green photosynthetic bacteria *Chlorobium limicola* and *Chloroflexus aurantiacus* were studied by measurements of flash-induced absorbance changes and fluorescence. Upon excitation with 35 ps, 532 nm flashes, large absorbance decreases around 750 nm were observed that were due to the disappearance of ground state absorption of the main pigment, bacteriochlorophyll (BChl) *c*. The absorbance changes decayed after the flash with a time constant of approx. 1 ns, together with faster components. Absorbance changes that could be ascribed to formation of excited BChl *a* were much smaller than those of BChl *c*. The yields of BChl *c* and BChl *a* fluorescence were measured as a function of the energy density of the exciting flash. At high energy a strong quenching occurred caused by annihilation of singlet excited states. An analysis of the results shows that energy transfer between BChl *c* molecules is very efficient and that in *C. limicola* excitations can probably move freely through the entire chlorosome (which contains about 10 000 BChls *c*). The chlorosome thus serves as a common antenna for several reaction centres. The small amounts of BChl *a* present in the chlorosomes of both species form clusters of only a few molecules. Upon cooling to 4 K the sizes of the domains of BChl *c* for energy transfer decreased considerably. The results are discussed in relation to recently suggested models for the pigment organization within chlorosomes.

Introduction

Most of the antenna system of green photosynthetic bacteria is contained in the so-called chlorosomes, oblong bodies about 100 nm long and 30–70 nm wide that are attached to the inner surface of the cytoplasmic membrane [1]. Light energy absorbed in the chlorosomes is transferred to the membrane, where the reaction centre and additional antenna pigments are situated [2]. The major pigments of the chlorosome are BChl *c* (or the related pigments BChl *d* or *e*) and carotenoid, but in addition the chlorosomes of the filamentous green bacterium *Chloroflexus aurantiacus* [3,4] and of the green sulphur bacterium *Chlorobium limicola* [5,6] were found to contain small amounts of BChl *a* that may serve to facilitate energy transfer to the membrane [6,7]. Models for the pigment organization of these chlorosomes have been proposed in which BChl *c* protein complexes form

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Abbreviation: BChl, bacteriochlorophyll.

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rod-like structures within the chlorosome envelope [5,8–10], while BChl *a* is supposed to be located near the site of attachment to the cytoplasmic membrane [5,8].

The present communication deals with the characteristics of energy transfer within these chlorosomes, studied by means of picosecond absorption difference spectroscopy and measurements of fluorescence quenching by excitation annihilation. The first technique allows a determination of the spectra and the kinetics of the absorbance changes due to the formation and decay of excited states of the antenna pigments [11]. Analysis of the data provides information about the interactions among the pigment molecules and about the pathways and rates of energy transfer, as has already been shown for membranes of several photosynthetic bacteria [12–14]. The second technique is based on the phenomenon of singlet–singlet annihilation and the concomitant quenching of fluorescence. This annihilation can take place when more than one excitation is present in a domain. Such a domain is defined as the ensemble of antenna pigment molecules between which energy transfer is not restricted by barriers. Whether such a quenching will indeed occur when two excitations are present in one domain depends on the ratio, r, of the rate of mono-excitation decay γ_1 (due to fluorescence and radiationless processes), and that of bi-excitation decay due to annihilation between a pair of excitations, γ_2:

$$r = \frac{2\gamma_1}{\gamma_2} \tag{1}$$

The parameter γ_2 is a function of the rate of energy transfer between neighbouring antenna molecules and the size of the domain [15]. Paillotin et al. [16] have derived the following expression to describe the fluorescence yield, ϕ, as a function of z, the average number of excitations generated in a domain.

$$\phi(z) = \phi(0) r \sum_{k=0}^{\infty} \frac{(-z)^k}{r(r+1)\ldots(r+k)} \frac{1}{k+1} \tag{2}$$

in which $\phi(0)$ is the fluorescence yield at low excitation energy when no annihilation occurs. By comparing an experimental curve with curves generated by a computer from Eqn. 2 the value of r is obtained, as well as the size of the domain involved. Note that for $r = 0$ (which is the case when the domains are small and energy transfer is efficient) Eqn. 2 reduces to

$$\phi(z) = \phi(0) \frac{1 - e^{-z}}{z} \tag{3}$$

Den Hollander et al. [15] have formulated a random walk model which allows calculation of the rate constant for energy transfer between neighbouring pigment molecules, the so-called hopping rate constant, k_h, as well as of the molecular rate constants for energy transfer from the antenna pigments to molecules which act as quenchers of the fluorescence.

The results to be reported here indicate that, at room temperature in chlorosomes of *C. limicola*, excitations are confined mainly to BChl *c* during and after a 35 ps laser flash, whereas in *Cfl. aurantiacus* the excitations spend some of their lifetime in BChl *a*. At high flash intensities extensive singlet-singlet annihilation occurs. In chlorosomes of *C. limicola* at room temperature the domain for energy transfer probably comprises the entire chlorosome; at 4 K the domains appear to be considerably smaller.

Materials and Methods

Chlorobium limicola f. *thiosulfatophilum* 6230 was grown as described in Ref. 17. After breakage of the cells by means of a French press, so-called 'medium density' and 'depleted' chlorosomes were purified by sucrose-gradient centrifugation as described in Ref. 5. They were suspended in 10 mM Tris buffer (pH 7.8). Unless otherwise indicated, 10 mM dithionite was added to remove non-fluorescent quenchers [6].

Chloroflexus aurantiacus strain J-10-fl was grown in medium D of Pierson and Castenholz [18]. The cells were grown at low light intensity to obtain a A_{740}/A_{866} ratio of about 20. Chlorosomes were prepared by sonication of broken cells and subsequent treatment with the detergent Deriphat, followed by purification according to Feick et al. [4]. The chlorosomes were suspended in Tris buffer (pH 8.0)/5 mM $MgCl_2$. For fluorescence measurements sucrose (final concentration 0.5 M)

and glycerol (55% v/v) were added to obtain clear samples upon cooling.

A frequency-doubled Nd-YAG laser was used as the source of 35 ps, 532 nm excitation flashes with maximum energy of about 10 mJ. For absorption difference measurements, the remaining infrared light was used to generate 35 ps white probe pulses. The experimental set-up is described in detail in Ref. 11. Fluorescence measurements were performed with the spectrofluorometer described in Ref. 19. The data obtained were analyzed using the theory described by Paillotin et al. (see Introduction) to calculate domain sizes.

From the shape and position of the quenching curve the number of incident photons at which on the average one excitation per domain is generated was determined [20]. This number, together with the absorption of the sample at the wavelength of excitation (532 nm), yielded the number of excitations generated in the sample by the flash. The number of BChl molecules excited in the sample was then calculated from the efficiency of energy transfer from pigments absorbing at 532 nm to BChl. The total number of BChl molecules in the sample was obtained from the absorbance and the extinction coefficient at the wavelength of maximum absorption. The ratio of the number of excited BChl molecules and the total number of BChl molecules directly yields the size of the domain. For measurements at 4 K, a shrinkage of 5% upon cooling was taken into account. In the calculation of the various rate constants for energy transfer, the random walk model of Den Hollander et al. [15] was applied.

Results

Flash-induced difference spectra

Absorption difference spectra of isolated chlorosomes of the green sulphur bacterium, *C. limicola*, obtained in the presence of 10 mM sodium dithionite upon excitation with 35 ps flashes at 532 nm and measured with 35 ps probe pulses are shown in Fig. 1. The spectra were measured with coinciding probe and excitation pulses (zero time, closed circles) and with a delay of 200 ps between the centres of the excitation and probe pulses (open circles). The intensity of the exciting pulses was 1.6 mJ/cm^2. As will be shown

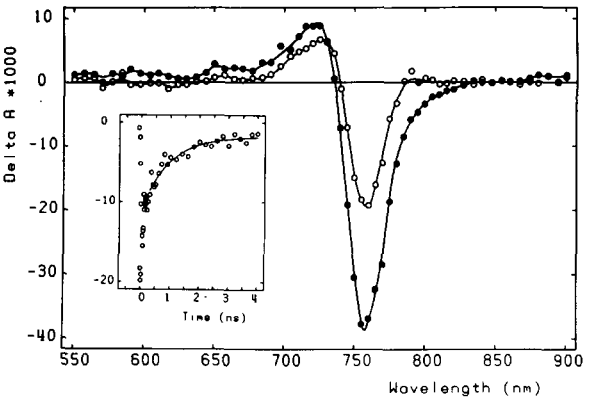

Fig. 1. Absorbance difference spectra of chlorosomes of *C. limicola* measured at room temperature in the presence of 10 mM sodium dithionite. The absorbance at 745 nm was 1.0 in a 2 mm vessel. Excitation with 35 ps, 532 nm flashes (1.6 mJ/cm^2). ●, at 0 ps, i.e., with coinciding probe and excitation pulses; ○, measured at 200 ps after the exciting flash. Inset: Kinetics of the absorbance change at 745 nm at an excitation energy density of about 0.9 mJ/cm^2. The solid line represents a fit for an exponential decay with time constant 1 ns.

below, at this intensity extensive excitation annihilation occurs. The predominant feature of both spectra is a bleaching centred at 758 nm that can be ascribed to the disappearance of ground-state absorption of BChl *c* upon formation of the excited singlet-state. Below 650 nm only small absorbance changes are observed, and there is little evidence for the formation of excited carotenoid, as was observed in membranes of green sulphur bacteria [12]. This indicates a very rapid decay of excited carotenoid, partly by energy transfer to BChl *c* [6], in part by other processes.

In the region 700–800 nm the difference spectra are fairly complicated. The absorption spectrum of the chlorosomes shows an absorption band of BChl *c* with a maximum at 749 nm [5]. The negative bands at 758 nm in the absorption-difference spectra are significantly narrower than the absorption band of BChl *c* and are located at about 9 nm longer wavelength. In addition, the spectra show positive bands around 720 nm. These features resemble those observed for antenna BChl *a* excitation in membranes of *Rhodospirillum rubrum* [13] and *C. aurantiacus* [14], and can be likewise explained by a bleaching of the Q_y absorption band of the excited BChl *c* molecule and

a simultaneous blue shift of neighbouring pigments. The recently determined primary structure of the BChl-*c*-binding protein [21] suggests that the BChl *c* molecules that bind to one protein are close enough to exhibit considerable exciton interaction, as is also indicated by the circular dichroism spectrum [22]. This may explain the complicated shape of the absorption-difference spectra.

It has recently been shown that chlorosomes of *C. limicola* contain a small amount of BChl *a* (about 1.2% of the total BChl content) with Q_y absorption maximum near 794 nm [5]. The difference spectrum obtained at zero time shows a weak shoulder around this wavelength that may be ascribed to excitation of this pigment. The amount of excited BChl *a* (BChl* *a*) appears to be small as compared to that of BChl* *c*, even if we take into account that the band at 758 nm is composed of bleaching of BChl *c* combined with a shift of the absorption band. This shift may well account for 50% of the amplitude of the difference spectrum at 758 nm [13]. Even if we allow for this correction, the ratio BChl* *a*/BChl* *c* is clearly smaller than the value of 2.2 that would be obtained if a Boltzmann distribution of excitations between BChl *c* and BChl *a* exists. This indicates that the losses in BChl *a* are much greater than in BChl *c*, so that the population of BChl* *a* will always be small, in agreement with the observation that only BChl *c* emission could be detected in the room temperature fluorescence spectrum [6].

The spectrum measured at 200 ps is different from that measured during the flash. The amplitude of the negative band indicates that at least 50% of BChl* *c* has decayed. However, the band is narrower and the positive band at 720 nm has shifted to 728 nm, while a small absorption increase on the long-wavelength side of the main bleaching has developed. This spectrum shows little evidence for the presence of BChl* *a*, and it appears that a bleaching near 800 nm, if any, has disappeared behind the absorbance increase in this region. This also suggests that the lifetime of BChl* *a* is shorter than that of BChl* *c*. As will be discussed below, there is no evidence that this effect is due to more extensive excitation annihilation in BChl *a* than in BChl *c*. That we observed relatively more BChl *a* bleaching at zero time than at 200 ps can be explained by direct energy transfer from carotenoid to BChl *a*. Such rapid energy transfer would initially create an extra amount of BChl* *a*. Thereafter, BChl *a* would be populated only via 'normal' energy transfer from BChl *c*. The excitation spectrum of 'depleted chlorosomes' from which BChl *c* had been removed [6] in fact provides evidence for such a direct pathway.

Fig. 1, inset, shows the kinetics of the absorbance changes at 745 nm. Immediately after the flash the decay was relatively fast, but it gradually slowed down. From about 300 ps onwards the kinetics could be fitted reasonably well by a mono-exponential curve with a lifetime (1/e) of 1 ns (solid line).

Fig. 2A shows absorbance difference spectra of 'depleted' chlorosomes of *C. limicola*, treated with 10 mM sodium dithionite. These chlorosomes have lost all, or practically all, BChl *c*, but have retained their BChl *a* and most of their carotenoid [5,6]. In addition, they contain a small amount of bacteriopheophytin *c*, absorbing at 670 nm [5,6]. The absorbance difference spectra were measured during the exciting flash (zero time) and after 200 ps. The main features of both difference spectra are bleachings at 670–675 and at 795–800 nm, which may be attributed to the disappearance of the ground-state absorption of bacteriopheophytin *c* and BChl *a*, respectively, upon formation of the excited states of these pigments. The differences in the locations of the maxima of the negative bands observed at zero time and at 200 ps are not significant in view of the limited accuracy of the experiment. In contrast to the results obtained with 'normal' chlorosomes, a large absorbance increase was observed centred at 570 nm which may be attributed to the formation of singlet-excited carotenoid. Similar increases were observed in bacterial membranes [12–14]. As shown by the spectrum measured at 200 ps, the excited state of carotenoid had largely disappeared after 200 ps.

The kinetics at 570 and 800 nm are shown in Figs. 2B and C. At 570 nm the time dependence of the initial increase and rapid decay was largely determined by the temporal profiles of the excitation and probe pulses, indicating that the lifetime of singlet-excited carotenoid is very short. At 800

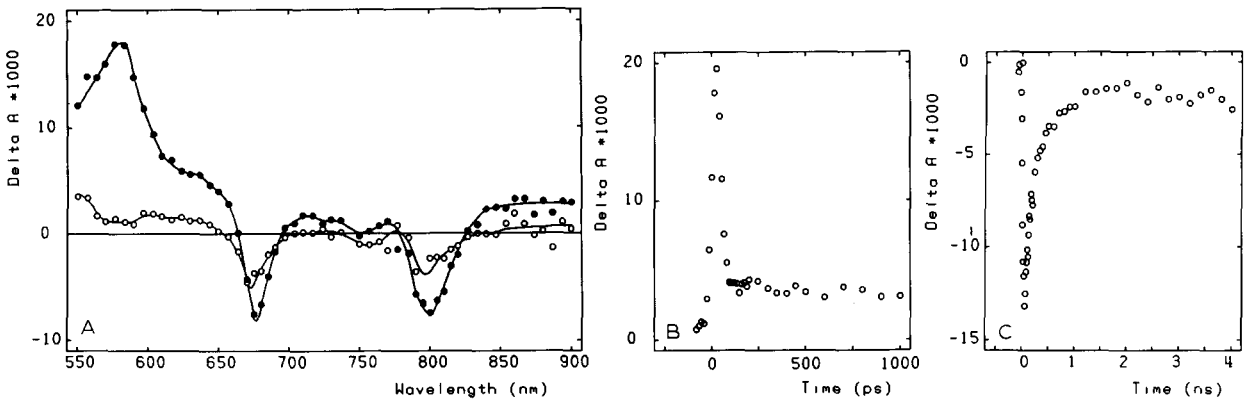

Fig. 2. (A) Absorbance difference spectra of so-called 'depleted chlorosomes' of *C. limicola*. Conditions as in Fig. 1. ●, 0 ps; ○, 200 ps. (B) and (C) kinetics at 570 (1.7 mJ/cm^2) and 800 nm (2.9 mJ/cm^2), respectively.

nm an initially rapid decay was observed, followed by a slower one that could be fitted by an exponential component with a time constant of 300 ps and a constant one. The constant components at 570 and 800 nm are probably due to the formation of triplet states of carotenoid and BChl *a*, respectively.

Flash-induced difference spectra of chlorosomes of the green filamentous bacterium *Cfl. aurantiacus* are shown in Fig. 3. In first approximation the spectra are similar to those obtained with *C. limicola*. However, the spectrum of *Cfl. aurantiacus* chlorosomes measured at zero time (closed circles) shows an absorbance increase near 590 nm that is probably caused by formation of singlet-excited carotenoid. This absorbance increase has largely disappeared after 200 ps. In the near-infrared region, the spectrum measured at 200 ps after the flash (open circles) is quite similar to that obtained with *C. limicola*, except that the maxima and minima are located at somewhat shorter wavelengths. The maximum bleaching was at 748 nm, i.e., at 6 nm longer wavelength than the maximum of the absorption spectrum (742 nm) [3,7]. As in *C. limicola* chlorosomes, the negative band is flanked by two positive bands, one near 712 nm and a weak one near 775 nm.

Chlorosomes of *Cfl. aurantiacus* contain about 4% BChl *a* (relative to BChl *c*), with a Q_y absorption band near 792 nm [3,4,7]. The contribution by BChl* *a* to the difference spectra at 790–800 nm is difficult to estimate, but appears to be even smaller than in chlorosomes of *C. limicola*. At first sight this is a rather unexpected result, since the emission bands of BChl *c* and BChl *a* in the fluorescence spectrum reported by van Dorssen et al. [7] have roughly the same intensity. However, as will be shown below, at the high excitation densities used the fluorescence intensity of BChl *a* is considerably lower than that of BChl *c*, presumably because of severe excitation annihilation in BChl *a*. This explains in part the observed discrepancy; in addition, one has to assume that the absorbance decrease due to BChl *a* excitation is nearly balanced by a fairly large absorbance

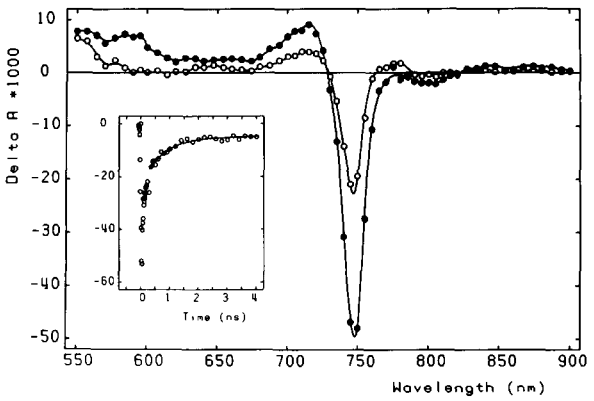

Fig. 3. Absorbance difference spectra of chlorosomes of *Cfl. aurantiacus*. Excitation with 35 ps, 532 nm flashes with average intensity of 1.2 mJ/cm^2. ●, 0 ps; ○, 200 ps. Inset: kinetics at 745 nm; excitation intensity 1.2 mJ/cm^2.

increase due to formation of BChl* c in this region.

Fluorescence quenching

Fig. 4 shows the time-integrated fluorescence yield of chlorosomes of *C. limicola* at room temperature upon excitation at 532 nm with 35 ps flashes, plotted as a function of flash energy. The fluorescence was detected at 780 nm, at the maximum of BChl c emission [6], both in the absence and in the presence of 10 mM sodium dithionite. For clarity, the curve in the absence of dithionite has been multiplied by a factor of 10. Both curves show a decrease of fluorescence yield due to singlet-singlet annihilation with increasing flash energy, but it can be seen that in addition to an increase in fluorescence yield by a factor of 10 (as has been reported before [6]), the presence of dithionite causes a large shift of the curve towards lower energy. Both curves could approximately be fitted with r values of at least 2 (see Introduction). Taking $r = 2$, the curve obtained in the presence of dithionite yields a domain size, N_D, of about 7500 BChl c molecules if an energy transfer efficiency from carotenoid to BChl c of 80% [7] and a specific in vivo extinction coefficient for BChl c of 102 $mM^{-1} \cdot cm^{-1}$ at 742 nm are assumed [7].

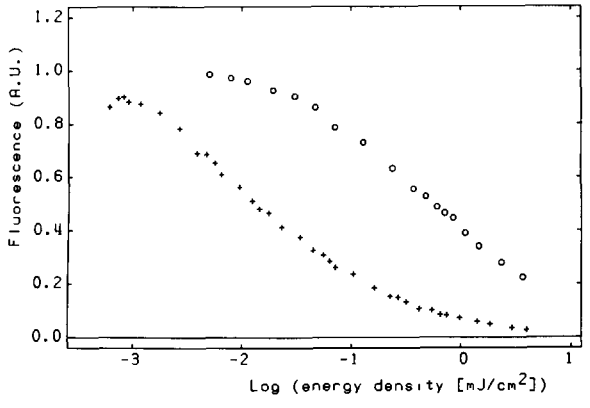

Fig. 4. Time-integrated fluorescence yield of chlorosomes of *C. limicola* at room temperature induced by 35 ps, 532 nm flashes, plotted as a function of the energy density of the exciting flash. For clarity, the averages of ten successive data points are plotted. O, in the absence of sodium dithionite, normalized at low energy density; +, in the presence of 10 mM sodium dithionite, reduced by a factor of 10. The fluorescence was measured at 780 nm. A.U., arbitrary units.

This domain size represents a minimum value, since for $r > 2$ the theoretical curves become very hard to distinguish. With $r = 3$ we calculate $N_D = 10\,000$. Since the chlorosome actually contains about 10 000 BChl c molecules [23], it is likely that energy transfer is not constrained within the chlorosome.

If we make the assumption that the efficiency of energy transfer from carotenoid to BChl c is the same in the sample which had not been treated with dithionite, we arrive at a minimum domain size of 145 BChl c molecules in the absence of sodium dithionite, using again the lower limit $r = 2$. It has been assumed that dithionite removes non-fluorescing quenchers [7]. These quenchers are therefore still active in this preparation and they should have an effect on the fluorescence yield, as well as on the value of r. Since the loss yield can be written as $\phi = k_1/\gamma_1$ [15] in which k_1 is the decay rate in the absence of additional quenchers, γ_1 must be 10-times higher for this preparation than for the sample which has been treated with dithionite. Then, because r is directly proportional to γ_1 (see Eqn. 1), the r value for the untreated sample must also be 10-fold higher. Considering only this effect and using an r value of 20–30 instead of of 2–3, we arrive at a domain size of 1000–1200. The apparent discrepancy will be discussed below.

Fig. 5 shows annihilation in chlorosomes of *C. limicola* in the presence of dithionite at 4 K. Detection was either at 785 nm, in the maximum of BChl c emission (open circles) or at 830 nm where BChl a fluoresces (open squares). For BChl c an r value of 1 was found, and a domain size of about 870 molecules, assuming 80% energy transfer from carotenoids to BChl c [6]. It thus appears that a strong decrease in domain size occurs upon cooling. A similar observation has been made for chromatophores of *R. rubrum* [20], where the domain size decreases from about 1000 to about 150 BChl molecules upon lowering the temperature. This decrease in domain size from a value close to the size of the complete chlorosome to a much smaller size probably reflects a partitioning of the domain by barriers which can easily be overcome by excitations at room temperature, but not at 4 K.

The curve measured at 4 K for BChl a emis-

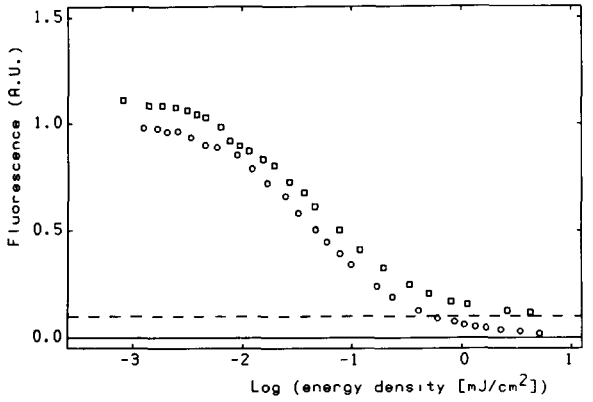

Fig. 5. Time-integrated, normalized fluorescence yield of chlorosomes of *C. limicola* at 4 K in the presence of 10 mM sodium dithionite. Horizontal scale: energy density of the 35 ps, 532 nm exciting flash. ○, BChl *c* emission at 785 nm; □, BChl *a* emission at 830 nm, shifted upward by 0.1 unit to enhance clarity.

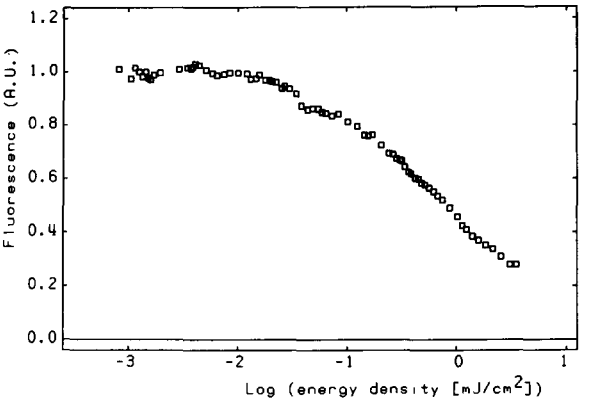

Fig. 6. Annihilation curve of depleted chlorosomes of *C. limicola* detected at 830 nm at room temperature, in the presence of 10 mM sodium dithionite.

sion was very similar to that for BChl *c*. (Fig. 5, upper trace). If we assume that most of the excitation energy reaches BChl *a* by energy transfer from BChl *c*, and neglect direct absorption and energy transfer from carotenoid, the emission yield of BChl *a* must be directly proportional to the number of excitations present in BChl *c*. Since 'back' transfer from BChl *a* to BChl *c* should be impossible at 4 K due to the large energy difference, the fluorescence yield of BChl *c* should not be affected by that of BChl *a*. Thus excitation annihilation between BChl *a* molecules should manifest itself by an extra quenching of BChl *a* fluorescence, and the absence of such quenching, as demonstrated by Fig. 5, indicates that no such annihilation occurs. From this one might conclude that the BChl *a* molecules are separated from one another. However, since the excitations do not arrive instantaneously in BChl *a*, but during the entire lifetime of BChl* *c*, annihilation effects may be reduced and we therefore conclude that the BChl *a* molecules form groups of not more than a few molecules.

Fig. 6 shows the annihilation curve of depleted chlorosomes. The experiment was performed at 4 K in the presence of 10 mM sodium dithionite. The efficiency of energy transfer from pigments absorbing around 532 nm to BChl *a* is about 30% [6]. A satisfactory fit of the quenching curve could not be found in this case; with $r = 0$, a domain of 7 connected BChl *a* molecules was obtained, in reasonable agreement with the results obtained with normal chlorosomes. No significant quenching of the bacteriopheophytin *c* emission at 685 nm could be observed.

Due to the very low fluorescence yield at room temperature (see below), measurements with chlorosomes of *Cfl. aurantiacus* did not yield accurate results at 298 K. Nevertheless, the results are of interest (Fig. 7), because in this case it was possible to compare the relative fluorescence yields of BChl *c* and BChl *a* at room temperature. At low

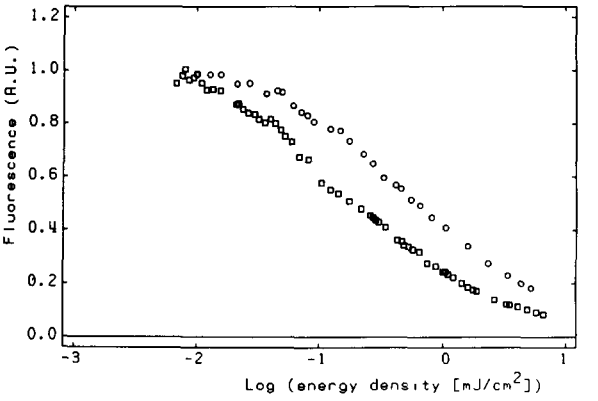

Fig. 7. Annihilation curves of chlorosomes of *Cfl. aurantiacus* at room temperature. ○, BChl *c* emission (765 nm); □, BChl *a* emission (810 nm). The curves are normalized at low excitation density (continuous light).

excitation density the yields of BChl *a* and BChl *c* were comparable, but at high flash energy a stronger quenching in BChl *a* was observed, indicating that considerable extra annihilation occurred in BChl *a* in these chlorosomes. For BChl *c* the same degree of quenching occurred at higher intensity than in *C. limicola* chlorosomes, suggesting that the domains may be smaller in this case. However, a determination of r was not possible with this material.

Fig. 8 shows the corresponding fluorescence curves at 4 K. The intensity dependence of BChl *c* emission can be fitted with r at least 2. Assuming $r = 2$ and an efficiency of 50% for energy transfer from pigments absorbing at 532 nm to BChl *c* [7], we find a domain size $N_D = 100$ connected BChl *c* molecules. At low temperature the curve obtained for the BChl *a* emission yield again did not coincide with that for BChl *c*, indicating that it reflects annihilation in BChl *a* itself, in addition to annihilation in BChl *c* prior to energy transfer to BChl *a*. For this preparation an accurate determination of the domain size of BChl *a* was possible. By the same reasoning as given for *C. limicola*, annihilation in BChl *c* will affect the curve measured for BChl *a* emission. Therefore, a correction was made by plotting the ratio of the two quenching curves versus the intensity. The result, which represents the true annihilation in BChl *a*, is shown as the broken line in Fig. 8. For this curve an r value of 0 is found. Together with an energy-transfer efficiency from BChl *c* to BChl *a* of 55% [7] and a

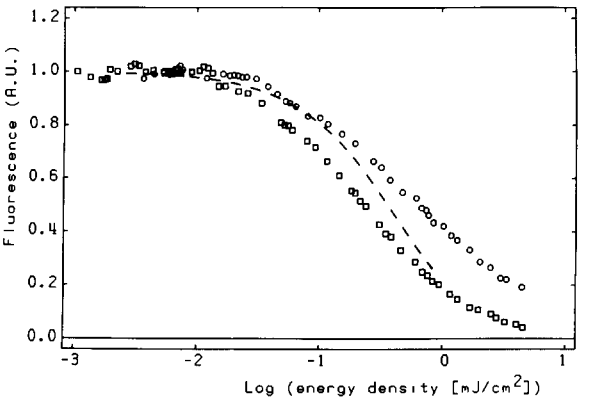

Fig. 8. Normalized annihilation curves of chlorosomes of *Cfl. aurantiacus* at 4 K. ○, BChl *c* emission (765 nm); □, BChl *a* emission (820 nm); — — —, annihilation curve for BChl *a* emission, corrected for annihilation in BChl *c* (see text).

pigment ratio of 30 BChl *c* per BChl *a* [4,7], a value of 2.3 for N_D is obtained, which indicates that on the average not more than 2–3 BChl *a* molecules are connected.

Rates of energy transfer

We will now proceed to analyse the data obtained so far to characterize the various energy-transfer processes.

By use of the random walk model of Den Hollander et al. [15], the rate constants for energy transfer between BChl *c* molecules (the so-called hopping rates, k_h) can be calculated from the domain sizes and the parameter r, provided the rates of mono-excitation decay γ_1 are known. In

TABLE I

CHARACTERISTICS OF ENERGY-TRANSFER PARAMETERS, LIFETIMES AND DOMAIN SIZES FOR BChl *c* IN CHLOROSOMES OF *C. LIMICOLA* AND *CFL. AURANTIACUS*

The fluorescence yields and lifetimes are given relative to those of chromatophores of *R. rubrum* with oxidized reaction centers. The symbols are explained in the text.

	T (K)	Fluorescence yield (rel.)	Loss yield (%)	Calculated lifetime (ps)	r	N_D	Hopping rate (s^{-1})
C. limicola	298	3.5	0.6	7	$\geqq 2$	$\geqq 145$	$\leqq 2.2 \cdot 10^{12}$
C. limicola + 10 mM sodium dithionite	298	20	3	40	$\geqq 2$	$\geqq 7500$	$\leqq 2.3 \cdot 10^{13}$
C. limicola + 10 mM sodium dithionite	4	200	30	400	1	870	$5.0 \cdot 10^{11}$
C. limicola 'depleted' + 10 mM sodium dithionite	298	70	12	140	0	7	–
Cfl. aurantiacus	298	1.7	0.27	3.5	–	–	–
Cfl. aurantiacus	4	12	1.9	25	$\geqq 2$	$\geqq 100$	$\leqq 4.3 \cdot 10^{11}$
R. rubrum chromatophores	298	100	16	200			

principle, values for γ_1 could be obtained from the decay kinetics presented in Figs. 1 and 3, but these kinetics are probably distorted by excitation annihilation. Also, very rapid decay components as recently observed (Ref. 24 and Gillbro et al., unpublished results) could not be detected with our apparatus. Therefore we measured the fluorescence yields of our preparations at room temperature and compared them to the yield of chromatophores of *R. rubrum*. Since for the lifetime of BChl* *a* in these chromatophores in the presence of closed reaction centres a value of 200 ps has been reported [25] and a loss yield of 16% [19], the lifetime of the BChl* *c* in our samples can then be calculated. The results are summarized in Table I. For chlorosomes of *Cfl. aurantiacus* the value for γ_1 at room temperature calculated in this way, although high, is not in disagreement with the upper limit of the fluorescence lifetime of 30–70 ps recently reported by Brune and Blankenship [24] from single photon counting experiments performed at low flash intensities. Using these data, the size of the domain ($N_D \geqslant 7500$) and the value of r ($r \geqslant 2$), Eqn. (14) from Ref. [15] yields $k_h \leqslant 2.3 \cdot 10^{13}$ s^{-1} for BChl *c* in chlorosomes of *C. limicola* at room temperature and in the presence of 10 mM dithionite. Since the pigments in chlorosomes are arranged in a three-dimensional structure [1], we used the Green's functions for three-dimensional lattices. For a domain of $N_D = 7500$ the value of this function is 1.45, as compared to 3.04 for the two-dimensional situation. (A value of $k_h \leqq 7.3 \cdot 10^{13}$ s^{-1} was obtained for a square lattice). An upper limit $k_h = 2.0 \cdot 10^{13}$ s^{-1} is obtained with $N_D = 10000$ and $r = 3$. In this calculation it is assumed that the domain is homogeneous, and that excitations move freely from BChl *c* to BChl *a* and vice versa.

At 4 K the domain size is smaller. Moreover, the fluorescence yield of BChl *c* is about 10-times larger than at room temperature, corresponding to a lifetime of approx. 400 ps. These data would yield $k_h = 5.0 \cdot 10^{11}$ s^{-1}.

These rates of energy transfer can be used to calculate the distance between two BChl *c* molecules by applying the well-known Förster equation [26]:

$$k_h = \frac{1}{\tau}\left(\frac{R_0}{R}\right)^6 \qquad (4)$$

in which $1/\tau$ is the lifetime of singlet excitations in the absence of nonradiative processes, and R_0^6 is proportional to the overlap integral between the emission band of the donor molecule and the absorption band of the the acceptor molecule. For the B800–850 complex of *Rhodobacter sphaeroides* at 4 K $R_0 = 5.3$ nm and $1/\tau = 5.6 \cdot 10^7$ s^{-1} [27]. A comparison of the overlap integrals for the B800–850 complex at 4 K [20] and BChl *c* in *C. limicola* indicated that R_0^6 must be a factor of 2 smaller for *C. limicola*, yielding $R_0 = 4.7$ nm. Together with $k_h = 6.9 \cdot 10^{11}$ s^{-1} this gives an (average) distance between BChl *c* molecules of 1.0 nm. This rather short distance agrees well with the high pigment density in these chlorosomes [1].

At 4 K there is presumably no excitation transfer from the BChl *a* back to BChl *c*. BChl *a* can therefore be regarded to function as a trap for excitations performing their random walk in the BChl *c* antenna. In order to be able to calculate the rate of this trapping, the loss rate constant k_l [15] must be known. The excitation spectrum for BChl *a* fluorescence indicates a high efficiency of energy transfer from BChl *c* to BChl *a* at low temperature [6]. Assuming a transfer efficiency of 70%, which would correspond to the loss yield of 30% as observed by us, and using the BChl *c* lifetime of 400 ps, we conclude that the intrinsic fluorescence lifetime of BChl *c* must be 1.3 ns, a number not unlike the lifetimes observed in other systems [28]. This corresponds to a loss rate $k_l = 7.5 \cdot 10^8$ s^{-1}. If we substitute this value, together with the loss yield of 30%, in Eqn. 12 of Ref. 15, we find a rate of energy transfer from BChl *c* to BChl *a* (trapping) $k_t = 1.9 \cdot 10^{11}$ s^{-1} if the BChl *a* molecules are separated, and $k_t = 1.2 \cdot 10^{12}$ s^{-1} if the BChl *a* forms groups of 5 molecules.

The domain for energy transfer (N_D) could not be determined for chlorosomes of *Cfl. aurantiacus* at room temperature. The low-temperature data suggest smaller domains (N_D at least 100) than for *C. limicola*. The fluorescence yield of BChl *c* in these chlorosomes at room temperature was found to be 1.7% of that of *R. rubrum* chromatophores, and increased 7-fold upon cooling to 4 K. This yielded a hopping rate constant k_h not greater than $4.3 \cdot 10^{11}$ s^{-1}. It was not possible to obtain realistic trapping rates in this case (see Discussion).

Discussion

The most important conclusion that can be drawn from the experiments reported here is that the domains for energy transfer in chlorosomes are very large. Our results obtained with chlorosomes of *C. limicola* indicate that at room temperature the transfer of excitations created in BChl *c* is not restricted by energy barriers, but that these excitations can move freely through the entire chlorosome, which contains about 10000 BChl *c* molecules [23]. A chlorosome thus functions as a common antenna for 5–7 reaction centres, and since energy transfer from the membrane back to the chlorosome appears to be possible [29], this common antenna may serve as an important factor in regulating energy transfer between photosynthetic units. At 4 K the domains for energy transfer are much smaller; apparently there are barriers for energy transfer within the chlorosome that are effective at low temperature, but are low enough as to be easily overcome at room temperature.

At 4 K the domains in chlorosomes of *Cfl. aurantiacus* are smaller than in *C. limicola*; unfortunately, we were not able to determine the domain size at room temperature in this case. For *Cfl. aurantiacus* it was possible to obtain an accurate measurement of the size of the BChl *a* domains at 4 K, which were found to be very small. The rate constant for energy transfer between Bchl *c* molecules (k_h) is in *C. limicola* at room temperature about $2.3 \cdot 10^{13}$ s^{-1}. Upon cooling to 4 K, k_h decreases about 30-fold.

It should be noted that for *C. limicola* in the absence of sodium dithionite and for *Cfl. aurantiacus* the lifetimes of BChl* *c* are shorter than the width of the exciting pulse. This will reduce the probability of singlet-singlet annihilation, and therefore cause an underestimation of the domain sizes. Thus the calculated hopping rates will also be too small. This may explain why no realistic trapping rates could be obtained in these cases.

At this point it should be stressed that our calculations are based on a homogeneous model for the arrangement of BChl *c* in the chlorosomes. However, the results of electron-microscopic studies [9,10] and the recent determination of the primary structure of the BChl-*c*-binding protein of *Cfl. aurantiacus* [21] clearly show that such a model is too simple. It is now thought that in *Cfl. aurantiacus* approx. 14 BChl *c* molecules are bound to a polypeptide dimer. These peptides are thought to be arranged in rods of about 50 Å diameter which are arranged in a parallel fashion along the long axis of the chlorosome. A basically similar structure probably applies to chlorosomes of *C. limicola* [10]. The intermolecular distance between the BChls *c* on the dimer are presumably small enough to cause considerable exciton interaction. This indicates that a more realistic model of the chlorosome would entail an ensemble of clusters, each consisting of about 14 BChls *c* among which essentially instantaneous energy transfer would occur. Energy transfer between the clusters would then be relatively slow. However, it can be shown that the use of such a model would not significantly modify the result of our calculation of the domain sizes. At the energy densities used for our calculation of domain sizes (approximately one excitation per 1000 BChl *c*) the probability that two excitations are generated in one cluster, and consequently the probability for annihilation within one cluster, is very low. With regard to energy transfer these clusters will therefore behave as single molecules. Taking into account that the extinction coefficient of a cluster of 14 BChl *c* molecules will be 14 times that of a single BChl *c* in vivo, the number of clusters per domain can be calculated in the same way as for the homogeneous model, and is consequently found to be 14-times smaller than the number of separate BChl *c* molecules.

The hopping rates between clusters will also be affected. Since these rates are proportional to the domain size, they will be reduced by a factor of 14. They are, however, also dependent on the Green's function of the domain size, but this function varies only very slightly with N_D for large domain sizes. The rates of energy transfer between clusters (k_h^c) thus calculated are for *C. limicola*: $k_h^c = 1.5 \cdot 10^{12}$ s^{-1} (298 K), and $k_h^c = 4.3 \cdot 10^{10}$ s^{-1} (4 K); for *Cfl. aurantiacus*: $k_h^c = 2.2 \cdot 10^{10}$ s^{-1} (4 K). It is of interest to note that these rate constants are in the same range as those derived from annihilation measurements with antenna systems of purple bacteria [20].

The flash-induced absorbance-difference spec-

tra show that in chlorosomes of *C. limicola* as well as in those of *Cfl. aurantiacus* the main bleaching that occurs at room temperature is due to formation fo singlet-excited BChl *c*, with much smaller contributions from excited BChl *a*. For both species the shape of the difference spectrum cannot be explained by the disappearance of the ground-state absorption of a monomeric BChl *c* molecule alone, but one has to assume a spectral shift of neighbouring pigments in addition. This supports the notion that considerable exciton interaction within a BChl *c* cluster occurs. The rate of decay of the bleaching is clearly slower than would correspond to the recently observed lifetimes of 30–70 ps (Ref. 24 and Gillbro et al., unpublished results) and to the low fluorescence yields observed by us. This applies especially to the approx. 1 ns component observed when exciton annihilation has ceased. Similar slow-decay components have been observed in the fluorescence decay curves of antenna systems of algae and higher plants [30,31]. It is not clear whether the slow components are due to isolation artifacts or if they reflect an essential inhomogeneity of the chlorosome.

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