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Energy transfer kinetics in chlorosomes from *Chloroflexus aurantiacus*: studies using picosecond absorbance spectroscopy

Mette Miller [1], Raymond P. Cox [1] and Tomas Gillbro [2]

[1] *Institute of Biochemistry, Odense University, Odense (Denmark) and* [2] *Department of Physical Chemistry, Umeå University, Umeå (Sweden)*

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We have investigated energy transfer in chlorosome preparations from the green photosynthetic bacterium *Chloroflexus aurantiacus* using picosecond absorbance spectroscopy. The observed energy transfer kinetics were similar in preparations obtained with the detergent Miranol and the chaotropic agent NaSCN. The results can be interpreted by a simple model in which energy transfer from bacteriochlorophyll *c* in the chlorosome to bacteriochlorophyll *a*-795 or *a*-808 takes 10 ps, and further transfer from these components takes 30 ps. Annihilation studies suggested that the excitation energy was restricted to a domain of about 30 bacteriochlorophyll *c* molecules. Time-resolved anisotropy decay measurements were also made and the results were used to make some structural predictions about the relative orientations of the various chromophores.

Introduction

The main light-harvesting pigment in many strains of green photosynthetic bacteria is bacteriochlorophyll *c* (BChl *c*) absorbing at 740–750 nm [1]. This is found in chlorosomes, which are oblong bodies attached to the cytoplasmic side of the cell membrane and surrounded by an envelope of lipids thought to be arranged in a monolayer. Freeze-fracture electron microscopy shows that the chlorosome is made up of rod elements extending the full length of the structure [2]. The diameter of these rod elements is 10 nm in the green sulphur bacterium *Chlorobium* [2,3] and 5–6 nm in the green filamentous bacterium *Chloroflexus* [4,5]. In both cases the rods appear to be made up from 6 nm subunits with a substriation of 2–3 nm [2,5].

In *Chloroflexus*, the chlorosome 'baseplate' contains a species of BChl *a* absorbing at 795 nm [6]. The attachment site of the chlorosome on the cytoplasmic membrane contains both the BChl *a* 808–866 antenna complex and the P865 reaction centre [7]. Steady-state and time-resolved fluorescence spectroscopy has confirmed the expected transfer of energy from BChl *c* in the chlorosome through the BChl *a* in the baseplate to the reaction centre in the membrane [8,9].

The exact organization of BChl *c* in the chlorosomes is still an open question. A 5.6 kDa protein has been isolated from chlorosomes from *Chloroflexus*. A role for this as a pigment-binding protein was suggested by Wechsler et al. [10], who proposed a model in which the rod elements of the chlorosomes were composed of dimers each with 14 BChl *c* molecules bound. However, it has recently been concluded that the 5.6 kDa protein has no role in pigment binding; the BChl *c* molecules in the chlorosome are envisaged as being organized in large aggregates similar to those observed in vitro in some non-polar organic solvents [11]. Interactions between the pigment molecules in the antenna would then depend only on pigment–pigment interactions, which might favour energy transfer over large domains.

The main aim of the experiments reported here was to obtain further information about energy transfer between the various antenna pigments in chlorosomes from the green photosynthetic bacterium *Chloroflexus aurantiacus* using picosecond absorbance spectroscopy and measurements of excitation energy annihilation, to complement previous measurements of picosecond fluorescence kinetics [12]. We have also measured time-re-

Abbreviations: BChl, bacteriochlorophyll; SDS, sodium dodecylsulphate.

Correspondence: M. Miller, Biokemisk Institut, Odense Universitet, Campusvej 55, DK-5230 Odense M, Denmark.

solved anisotropy changes to obtain information about the structural organization of the different types of antenna pigment.

A second aim of these experiments was to investigate possible effects of different methods of chlorosome isolation on the energy transfer properties. Methods have been described for isolation of chlorosomes using detergents and chaotropic agents [6,13–15]. These preparations might contain different amounts of 'baseplate' structures and be contaminated to different degrees by antenna complexes and reaction centres derived from the cytoplasmic membrane attachment site. Detergents might also disrupt the structure of the chlorosome and thus influence pigment–pigment interactions. We therefore decided to compare chlorosomes isolated using Miranol [6], the most commonly used detergent, with those isolated using the chaotropic agent, NaSCN [14].

A preliminary account of some of these results has already been presented [16].

Methods

Chlorosome preparation

Chloroflexus aurantiacus strain OK-70fl was kindly provided by Prof. J.G. Ormerod, University of Oslo. The cells were grown at 55°C in D medium salts [17] supplemented with 5 g/l sodium acetate trihydrate, 2 g/l casamino acids (Difco), 1 g/l yeast extract (Difco) and 0.6 g/l NH_4Cl and buffered with 10 mM Tris-HCl (pH 8.0) (Ormerod, J.G., personal communication). Cultures were grown in in 1.0 litre bottles completely filled with medium. The bottles were incubated on a rotary shaker at 150 rev/min and illuminated with a single 18 W fluorescent tube (distance of closest approach, 2.0 cm). The A_{740}/A_{805} ratio of the harvested cells was around 4.4.

Chlorosomes were prepared from freshly harvested cells either by the use of the detergent Miranol S2M-SF as described by Feick and Fuller [6] or by using the chaotropic agent NaSCN as described by Gerola and Olson [14]. The chlorosome preparation was stored frozen at −80°C.

SDS treatment of chlorosomes was performed as decribed in Ref. 18.

Picosecond spectroscopy measurements

Picosecond absorbance recovery measurements were performed at room temperature (about 23°C) using the pump-probe technique as described previously [19]. Light pulses were produced by a cavity-dumped, synchronously pumped dye laser at a frequency of 820 kHz. The excitation source of the dye laser was a mode-locked Nd-YAG laser (Spectra Physics). The dye DCM was used at 740 nm and Styryl 8 at 800 nm. The typical pulse width was 8 ps. The intensity of the laser pulse could be reduced by inserting neutral-density filters with absorbance values between 0.3 and 1.7 in the exciting beam. The chlorosomes were resuspended in 10 mM Tris-HCl (pH 8.0) at a concentration corresponding to an absorbance (1 cm light path) of between 5 and 10 at 740 nm. The measuring cell had a pathlength of 1 mm and was rotated during the measurements to avoid accumulation of long-lived intermediates in the excitation beam.

The angle between the polarization of the exciting light and the probe light was controlled by a Soleil-Babinet compensator situated in the excitation beam. Anisotropy measurements were made with the exciting light polarized parallel ($I_{\parallel}$) or perpendicular ($I_{\perp}$) to the analyzing light. Isotropic measurements were made with the polarization set to 54.7°, the magic angle. From these data the anisotropy, $r(t)$, is calculated as

$$r(t) = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$$

The recorded kinetic traces were fitted using a modified Marquardt algorithm to a sum of two or three exponentials convoluted with a Gaussian function representing the picosecond pulse.

Steady-state spectroscopy

Absorbance spectra were measured using a Perkin-Elmer 330 recording spectrophotometer, and steady-state fluorescence spectra were measured on a Spex Fluorolog 112 fluorometer with a Hamamatsu R 928 red-sensitive multialkali photocathode. Anisotropy was measured using standard techniques with polarizers in the excitation light beam and in front of the emission monochromator. Corrections were made for instrumental sensitivity factors for light with different polarizations [20].

Results

Composition of chlorosome preparations

Fig. 1 shows absorbance spectra of *Chloroflexus* chlorosomes isolated using either 0.3% (w/v) Miranol or 2 M NaSCN. The spectra have been normalized to the same absorbance at 740 nm. The same batch of cells was used for both preparations so that any difference in the observed pigment composition was the result of the preparation methods. The chlorosomes prepared with Miranol had a lower content of BChl *a*-795 and the BChl an 808–866 antenna complex as judged from the lower absorbance at around 800 nm and 870 nm. There was also less carotenoid in the preparation obtained using Miranol.

It has been reported that chlorosomes from *Chloroflexus* prepared under strictly anoxic conditions show a higher ratio of absorbance A_{450}/A_{740}, and a more efficient quenching of the BChl *a* fluorescence, than chlo-

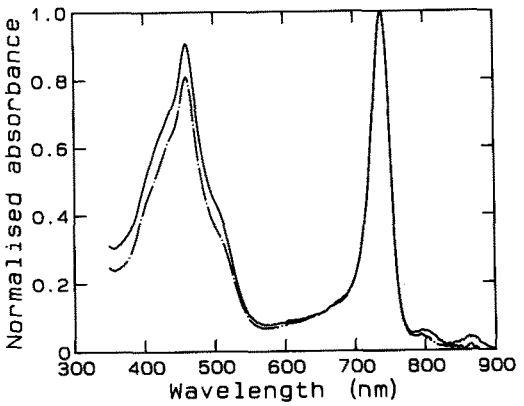

Fig 1. Absorbance spectra of *Chloroflexus* chlorosomes isolated using the detergent Miranol (broken line) or the chaotropic agent NaSCN (continuous line). The two spectra have been normalized to the same absorbance value at the peak at 740 nm.

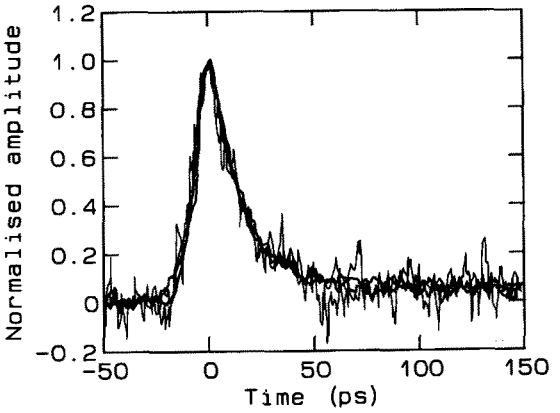

Fig. 2. Superimposed traces of the absorbance changes in chlorosomes at 750 nm induced by a laser pulse at the same wavelength. The chlorosomes were prepared using Miranol. The measurements were made with neutral density filters with absorbance values of 0.3, 0.7 and 1.0 and without a filter. The traces have been corrected for excitation intensity by dividing the signal intensity by the absorbance of the filter.

rosomes prepared without precautions [21]. However, when we isolated our chlorosomes using solutions degassed with argon (and also containing 10 mM dithionite in the case of a preparation using Miranol), we could not detect any change in the absorbance or fluorescence emission spectra of the isolated chlorosomes on exposure to oxygen (results not shown).

Annihilation study

When using excitation energies such as those used in these measurements (typically 10^{14} photons cm^{-2} $pulse^{-1}$) it is expected that more than one BChl molecule out of the large number in a chlorosome will be excited. This could in some cases lead to annihilation of excitation energy [22], which would lead to effects of flash intensity on the fluorescence and absorbance decay constants and a decrease of the fluorescence quantum yield.

When isolated chlorosomes were excited at 750 nm, a strong absorbance decrease was observed upon probing at the same wavelength, as a result of depletion of the ground state and formation of the excited state of BChl *c*. Fig. 2 shows a number of representative traces of the absorbance recovery kinetics in *Chloroflexus* chlorosomes prepared with Miranol. The traces were measured at 750 nm using different excitation intensities, with the signal size corrected for the excitation intensity. The figure demonstrates that changing the excitation intensity by an order of magnitude had no discernable effect on the fast lifetime.

In Fig. 3, the corrected signal amplitudes for experiments of this type are plotted against the log of the pulse intensity. As discussed by Paillotin et al. [22], the data can be analyzed using the expression:

$$\theta_F = \theta_F^0(1 - e^{-z})z^{-1}$$

where θ_F^0 is the fluorescence yield at low excitation energy when no annihilation occurs, and z is the number of hits per domain. This equation is valid when the bimolecular annihilation rate is much larger than the monomolecular decay rate [22]. This must be the case for the chlorosomes studied here, since no change in the excited state lifetime with excitation energy could be observed with the time resolution of our apparatus (about 2 ps), as demonstrated in Fig. 2. Thus the extremely fast annihilation within a domain of BChl *c* leads only to a decrease of the signal amplitude at the first time point observed, due to relaxation of the annihilitated molecules back to the ground state. This fast process does not effect the measured lifetime, which represents energy transfer from domains with a single remaining excited BChl *c* chromophore.

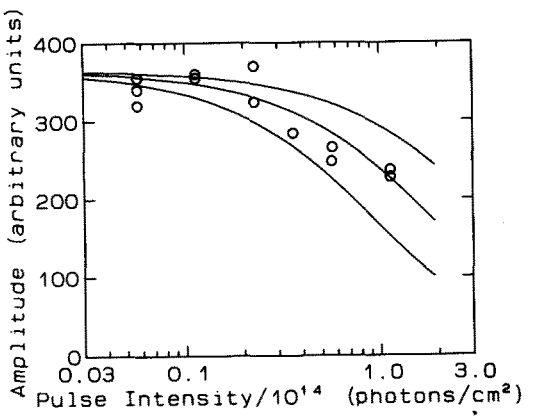

Fig. 3. Effect of pulse intensity on the amplitude of the absorbance change at 750 nm induced by a laser pulse at the same wavelength. The pulse intensity was varied using neutral density filters. The circles show the experimental values, and the lines show the expected results for domain sizes of 60 (upper curve), 30 and 15 BChl molecules, calculated as described in the text.

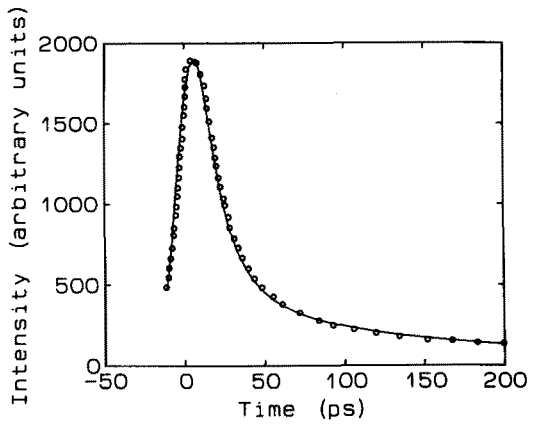

Fig. 4. Absorbance recovery kinetics of excited BChl *c* at 750 nm measured with the excitation pulse polarized at the 'magic angle'. The continuous line shows the fitted curve through the digitized experimental absorbance values. The chlorosomes were prepared using NaSCN.

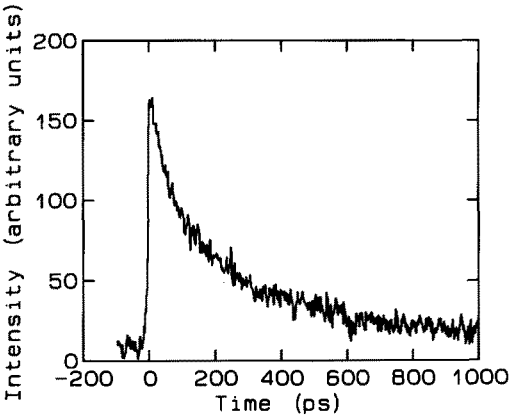

Fig. 5. Absorbance recovery kinetics of BChl *a* at 800 nm measured with the excitation pulse polarized at the 'magic angle'. The chlorosomes were prepared using Miranol.

The solid lines in Fig. 3 represent the expected curves for domain sizes of 15, 30 and 60 BChl *c* molecules. The results suggest that the excitation energy is not free to move over the whole chlorosome; rather, its movement is restricted by the structural organization of the BChl *c*. Vos et al. [23] reached rather different conclusions with *Chlorobium*, where they found that at room temperature the excitation was free to move over the whole chlorosome. However, they were unable to obtain corresponding results for *Chloroflexus*.

Energy transfer kinetics

On excitation of the main absorbance band at 750 nm due to BChl *c*, a very fast absorbance recovery is observed. In Fig. 4 we have fitted the experimental curve to a sum of three exponentials. The fast transient, which has a lifetime of 11 ± 2 ps, comprises about 80% of the total signal intensity. The second component, which comprises 15–20% of the signal, has a decay time of about 40 ps. More slowly decaying components make up less than 5% of the signal.

Fig. 5 shows an example of an experiment performed at 800 nm, where BChl *a* in the baseplate is preferentially excited. With this wavelength a satisfactory fit to the experimental data was obtained with a sum of two exponentials, giving two components of comparable amplitude. The fast decay has a lifetime of about 30 ps and the lifetime of the slow component is about 200–300 ps.

Table I shows the collected results for the lifetimes and relative amplitudes for the relaxation of excited bacteriochlorophyll in *Chloroflexus* chlorosomes prepared with either Miranol or NaSCN. It is noteworthy that the results obtained with the two chlorosome preparations are identical within experimental error.

Table I also includes the results of measurements on a sample of *Chloroflexus* chlorosomes treated with SDS. This preparation was completely devoid of BChl *a*, judged from the absorbtion spectra and from the lack of fluorescence emission at 803 nm (results not shown).

TABLE I

Lifetimes and relative amplitudes for the absorbance recovery kinetics of excited bacteriochlorophyll in different chlorosome preparations from Chloroflexus aurantiacus

The relative amplitudes and time constants were determined from digitised experimental curves as described in the Methods section. The values are means and standard deviations of the number of experiments given. SCN, chlorosomes prepared with NaSCN; Miranol, chlorosomes prepared with the detergent Miranol; SDS, chlorosomes prepared with NaSCN and treated with SDS.

Sample	Wavelength (nm)	n [a]	Lifetime (ps)			Amplitude (%)		
			T_1	T_2	T_3	A_1	A_2	A_3
SCN	750	6	10.8± 1.7	39± 11	350±200	76± 8	19± 8	5±3
Miranol	750	3	9.4± 1.2	26± 1	380± 70	79± 4	15± 4	6±1
SDS	750	2	8.8± 1.0	57± 6	520±130	79± 0	12± 2	10±1
SCN	800	8	31 ± 8	190± 70		46± 2	54± 3	
Miranol	800	3	43 ±23	280±160		44±11	56±11	

[a] Number of measurements.

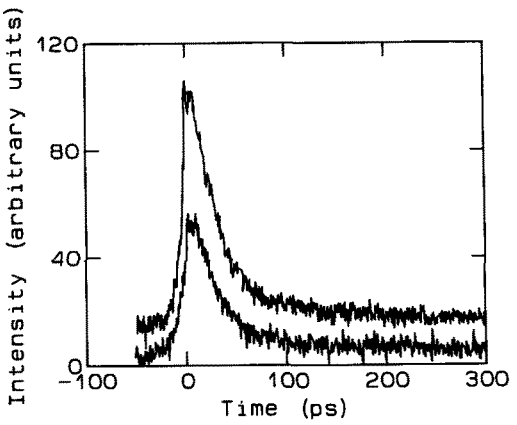

Fig. 6. Absorbance recovery kinetics of BChl *a* at 750 nm measured with the excitation pulse polarized parallel (larger signal) or perpendicular (smaller signal) to the measuring beam. The chlorosomes were prepared using NaSCN.

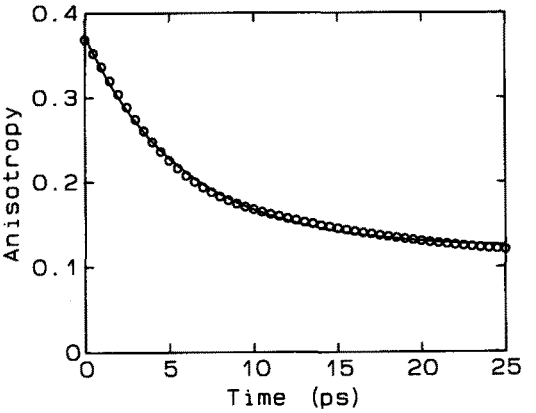

Fig. 7. Anisotropy decay kinetics of BChl *c* at 750 nm. The experimental points were calculated as described in the text from a pair of traces of the type shown in Fig. 6. The continuous line shows the fitted curve corresponding to a single exponential decay to a constant value. The chlorosomes were prepared using Miranol.

The energy transfer kinetics did not differ significantly from the two preparations of chlorosomes containing BChl *a*. It has been reported that treatment with SDS removes some of the chlorosome proteins [11,18]. Obviously, the depletion of BChl *a* and proteins did not interfere with the relaxation reactions of excited BChl *c* in our experiments.

Anisotropy decay kinetics

An example of measurements with parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) polarization at 750 nm is shown in Fig. 6. Analysis of the kinetics shows that there is an initial decay from the theoretical maximum anisotropy of 0.4 to a value of 0.2 ± 0.05 in less than 10 ps (Table II). There was no corresponding kinetic component in our absorbance decay measurements.

When the polarization experiments were performed at 800 nm (Fig. 7) we could resolve a fast relaxation (7.6 ps) of the anisotropy from $r = 0.41$ to $r = 0.13$ (Table II). This does not correspond to any isotropic absorbance changes.

TABLE II

Anisotropy decay kinetics in chlorosome preparations

Anisotropy values were calculated from the digitized traces from pairs of experiments in which the measuring beam was polarized parallel or perpendicular to the exciting pulse. Each value is the mean and standard deviation of at least five measurements. The results for chlorosomes prepared with Miranol and with NaSCN were indistinguishable and have been combined. The time constant and r_∞ values for the measurements at 800 nm were determined by a least-squares fit to a single exponential decay to the r_∞ value.

Wavelength (nm)	r_0	r_∞	T_1 (ps)
750 [a]		0.20 ± 0.05 [b]	<10
800	0.41 ± 0.05	0.13 ± 0.02	7.6 ± 1.7

[a] r_0 could not be determined.
[b] Determined after 40 ps.

Steady-state fluorescence anisotropy

Excitation of BChl *c* at 690 nm gave a fluorescence emission spectrum with maxima at 754 and 803 nm as shown in Fig. 8. The same result was obtained with chlorosomes prepared with either NaSCN or Miranol. It seems reasonable to assume that the two maxima arise from the BChl *c* in the chlorosome rods and the BChl *a*-795, respectively. This result shows that there must be some energy equilibration between the two pigment pools. No fluorescence emission at 803 nm was observed in the SDS-treated preparations, in agreement with the results of Brune et al. [18] (results not shown). In view of the low sensitivity of the apparatus used at wavelengths above 850 nm we would not observe a low level of emission at 880 nm due to BChl *a*-866 in the

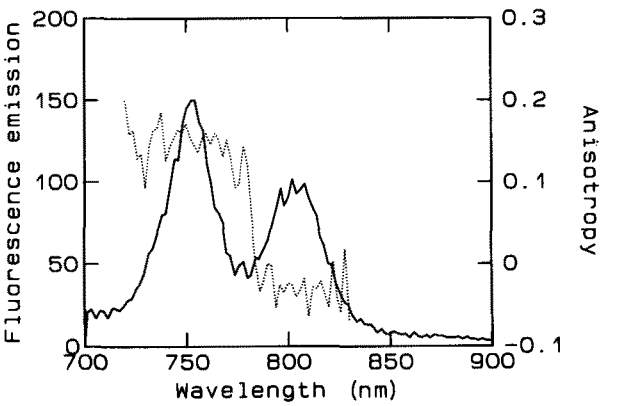

Fig. 8. Steady-state fluorescence emission spectra of chlorosomes prepared using NaSCN. Excitation was at 690 nm and the spectrum has not been corrected for detection system sensitivity factors. The continuous line shows the fluorescence emission measured with the polarizer set at the 'magic angle'. The broken line shows the anisotropy calculated as described in the text.

uncorrected spectra, although the absorbance spectrum (Fig. 1) shows that this is present.

In order to study the angular distribution of the bacteriochlorophyll Q_y transitions, we measured the fluorescence anisotropy of the two emissions. This was 0.18 around 750 nm and close to zero around 800 nm (see broken line in Fig. 8). Within the experimental error, the anisotropy observed for the BChl *c* band is the same as the corresponding absorbance anisotropy at times greater than 40 ps, after the fast component in the absorbance anisotropy decay was completed (see Table II).

Discussion

Comparison of chlorosomes prepared using different procedures

The results reported here show that the kinetic and spectroscopic properties of chlorosomes isolated from *Chloroflexus aurantiacus* using the detergent Miranol or the chaotropic agent NaSCN are the same and therefore independent of the isolation procedure. The chlorosomes prepared by either method contained proteins in the range between 5 to 18 kDa (results not shown). However, in the SDS-treated chlorosomes which are completely devoid of BChl *a* and are partly depleted of proteins [18], particularly the 5.6 kDa protein [11], the same energy transfer kinetics for BChl *c* were observed (see Table I). This suggests that removal of proteins has little effect on the organization of the BChl *c* molecules in the chlorosomes.

Kinetics of energy transfer

Analysis of our absorbance decay kinetics gives lifetimes which correspond to the following minimum kinetic scheme:

$$\text{BChl } c \xrightarrow{10\text{ ps}} \text{BChl } a_{795} \xrightarrow{30\text{ ps}} \text{BChl } a_{808-866}$$

$$\rightarrow \text{decay products}$$

where the species absorbing at 800 nm includes BChl *a* in the chlorosome (baseplate) and in the cytoplasmic membrane (attachment site). Although the results presented here do not provide unequivocal evidence that energy acceptor in the 30 ps step is the BChl a 808–866 complex, this seems plausible. The presence of active reaction centres in these preparations is supported by the results of experiments at 800 nm in which redox poising was used to open and close the centres, and the observation of absorbance changes when pump-probe measurements were performed at 870 nm (results not shown).

These lifetimes are in good agreement with those reported from fluorescence measurements. Mimuro et al. [9] measured the lifetimes of excited antenna pigments in whole cells of *Chloroflexus* using time-resolved fluorescence spectroscopy and obtained lifetimes of 16 ps for BChl *c*, 41 ps for BChl *a*-808 and 250 ps for BChl *a* 808–866. Holzwarth and co-workers [24] made measurements of fluorescence lifetimes in isolated chlorosome preparations from *Chloroflexus* and observed a major component of 14 ps for BChl *c*, with smaller amplitude components of 30–50 ps and about 450 ps.

It is somewhat surprising that we observed similar kinetics both in the two chlorosome preparations containing BChl *a* and in the SDS-treated chlorosomes apparently devoid of BChl *a* [18]. However, the pigment content of this material has been determined only by spectroscopic techniques which would not necessarily detect a small fraction of BChl *a* or BChl *c* acting as an efficient acceptor of excitation energy, especially if this had altered properties leading to a low fluorescence yield. The significance of this result cannot be determined until the pigment contents have been determined by chromatography.

In chlorosomes depleted of BChl *a* by detergent treatment followed by gel electrophoresis, Holzwarth et al. [24] were able to resolve a 5 ps component for BChl *c* showing a negative amplitude at higher wavelengths, in addition to the 14 ps and slower components. It was suggested that this fast component was due to the energy transfer between two different BChl *c* pools in the antenna or a relaxation process in excitonically coupled BChl *c* aggregates. We were not able to detect this very fast component by absorbance measurements in *Chloroflexus* chlorosomes treated with SDS (see Table I), although this would have been just within the time resolution of our measuring system. However, experiments at wavelengths less than 730 nm would be necessary to eliminate the possibility of transfer between two spectrally different BChl *c* pools.

It is worth noting that in *Chlorobium limicola* the transfer lifetime for BChl *c* → BChl *a* has been found to be about twice as long as for *Chloroflexus* chlorosomes, i.e., 20–30 ps [25,26].

Organization of BChl c in the chlorosome

There are at present two competing models for the organization of BChl *c* in the chlorosome. In one model the pigment is associated with the 5.6 kDa chlorosome protein, which is thought to make up the rod elements observed by electron microscopy [10]. In the alternative model [11], protein is relegated to a subsidiary role, and the rods are thought to consist of BChl aggregates in which the individual BChl molecules are arranged in a shallow helix forming a cylinder similar to that observed by Worcester et al. [27] in octane/toluene mixtures. This model of chlorosome structure with direct pigment–pigment interactions between bacteriochloro-

phyll molecules is in agreement with the results of earlier spectroscopic experiments [28–32].

The observation that the 5.6 kDa protein is associated with the chlorosome envelope (Wullink, W., personal communication) and can be removed from the chlorosomes by SDS without detectable effects on energy transfer (Table I and Ref. 24) provides strong evidence supporting a model based on pigment–pigment interactions, although not necessarily with a close resemblance to the model systems of Worcester et al. [27]. Our estimate of a domain size of about 30 molecules suggests that the excitation energy cannot be delocalized over the whole aggregate; instead it is possible that the domain size corresponds to the number of BChl molecules in ring corresponding to a single turn of the helix in a 'Worcester cylinder'. The diameter of the rod elements observed by electron microscopy in *Chlorobium* is 11 nm [2], in good agreement with the diameter of a 'Worcester cylinder' of BChl *c*. The agreement with the diameter of the rods observed with *Chloroflexus* is less good; however, it is possible that the arrangement of BChl in the aggregates is influenced by proteins, which could also contribute to the substriations observed by electron microscopy in freeze-fractured preparations [5]. It may also be significant that BChl *c* is not one chemical species but consists of a mixture of homologues with various side-chains attached to the 4 and 5 positions [28]; the relative proportions of the different homologues is different in different green bacteria [28,33]. These side-groups do not affect the spectral properties of the molecules, but are known to influence the the formation of aggregates in three nonpolar solvents [34] and might well influence the organization of other types of aggregate.

Our results on BChl *c* anisotropy changes can be used to make some structural predictions. If one assumes that the BChl *c* molecules are distributed symmetrically around the rod axis, approximating a 'Worcester cylinder', and that there is a very fast randomization of the excited states between the BChl *c* molecules in a domain, the anisotropy at long times-should give the order, *S*, of the BChl *c* molecules in the chlorosome [35]

$$r = r_0 S^2, \text{ where } S = (3\cos^2\theta - 1)/2$$

From this expression θ, the average angle between the BChl *c* Q_y transition and the long symmetry axis of the rod, can be calculated from the results in Table II. Inserting $r_0 = 0.40$ (theoretical limit) and $r = 0.20$ we obtain $\theta = 22 \pm 5°$ (or $\theta = 158 \pm 5°$). This result indicates that the mean orientation of the strong BChl *c* transition is close to the rod symmetry axis.

These predictions can be compared with the results of linear dichroism measurements. Betti et al. [36] and Van Dorssen et al. [8] reported a value of about 40° for the angle between the long axis of the chlorosome and the Q_y transition moment for BChl *c* which lies in the plane of the porphyrin-ring system. However, in a recent study using chlorosomes oriented either in electric fields or in polyacrylamide gels, van Amerongen et al. [37] obtained a value of 20° between the Q_y transition moment of BChl *c* and the long axis of the chlorosome. This value is in agreement with the one which can be calculated from our anisotropy data.

Fetisova et al. [38] have reported a somewhat higher value ($r = 0.32$) for the time-resolved BChl *c* fluorescence anisotropy for both *Chloroflexus* and *Chlorobium* chlorosomes. The reason for the discrepancy between this value and our results for steady-state fluorescence anisotropy is uncertain.

Structural relationship between BChl c and BChl a

The organization of BChl a within the chlorosome, and its relationship to the BChl *c*, remains uncertain. At 800 nm we observed a fast anisotropy relaxation of BChl *a* which is not observed in the corresponding isotropic kinetic traces. This may result from transfer between BChl a molecules within a 'baseplate' complex. The ordering of the BChl *a* Q_y band is clearly not in the membrane plane or perpendicular to it because this would give $r = -0.2$ or 0.4, respectively, and not the observed value of 0.13 ± 0.02. If a symmetric distribution about the normal to the membrane plane is assumed, the calculated value of θ is 32°.

The steady-state fluorescence anisotropy at 800 nm is lower than the value obtained from corresponding absorption data. This indicates that there is a further loss of anisotropy (order) as the energy is transferred from BChl *c* to BChl *a*, as expected if the two systems are not ordered in the same way about the same symmetry axis. If the symmetry axis of the BChl *c* molecules in the aggregate is taken to lie along the membrane plane and the the angle between the BChl a Q_y transition and this plane is taken as 58° (90°–32°), we can calculate a value of −0.02 for the anisotropy of the BChl *a* fluorescence as the energy is transferred from the BChl *c* of the rods to BChl *a* in the membrane. This agrees well with the experimental data.

Conclusions

Our kinetic results are compatible with a model in which the functional unit of BChl *c* organization is comprised of a number of interacting molecules arranged in a structure similar but not identical to that observed in solution. One limitation is that the functional domain size appears to be much smaller than the total number of BChl *c* molecules in a chlorosome. A comparison of the kinetic and spectroscopic properties of BChl *c* aggregates in aqueous suspensions and intact *Chloroflexus* chlorosomes is now in progress to provide further information on these issues.

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