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Antenna Organization in Green Photosynthetic Bacteria. 2. Excitation Transfer in Detached and Membrane-Bound Chlorosomes from *Chloroflexus aurantiacus*†

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ABSTRACT: The photosynthetic antenna of *Chloroflexus aurantiacus* includes bacteriochlorophyll (BChl) c_{740} and BChl a_{792}, both of which occur in chlorosomes, and B808-866 (containing BChl a_{808} and BChl a_{866}), which is membrane-located (subscripts refer to near-infrared absorption maxima in vivo). BChl a_{792} is thought to mediate excitation transfer from BChl c_{740} to BChl a_{808}. Lifetimes of fluorescence from BChl c_{740} and BChl a_{792} were measured in isolated and membrane-bound chlorosomes in order to study energy transfer from these pigments. In both preparations, the lifetime of BChl c_{740} fluorescence was at or below the instrumental limit of temporal resolution (about 30–50 ps), implying extremely fast excitation transfer from this pigment. Attempts to disrupt excitation transfer from BChl c_{740}, either by conversion of part of this pigment to a monomeric form absorbing at 671 nm or by partial destruction of BChl a_{792} by oxidation with $K_3Fe(CN)_6$, had no discernible effects on the lifetime of BChl c_{740} fluorescence. Most (usually >90%) of the fluorescence from BChl a_{792} decayed with a lifetime of 93 ± 21 ps in membrane-attached chlorosomes and 155 ± 22 ps in isolated chlorosomes at room temperature. Assuming that the only difference between these preparations is the occurrence of excitation transfer from BChl a_{792} to B808-866, a 41% efficiency was calculated for this process. This value is lower than the 60% efficiency of excitation transfer from BChl c_{740} to B808-866 determined by comparison of fluorescence excitation and absorption spectra of membranes with attached chlorosomes and compares even less favorably with the 100% efficiency of excitation transfer found in whole cells by the same method. Furthermore, measurements at 77 K (on different samples) did not show an increased lifetime of BChl a_{792} fluorescence when isolated chlorosomes were compared with membrane-bound chlorosomes. These results imply either that BChl a_{792} is not an obligatory intermediate in energy transfer from BChl c_{740} to B808-866 or (more probably) that chlorosome isolation introduces new processes for quenching fluorescence from BChl a_{792}.

The light-harvesting antenna in the green photosynthetic bacterium *Chloroflexus aurantiacus* includes both a membrane-attached structure (the chlorosome) with about 10 000 bacteriochlorophyll (BChl) *c* and 350 BChl *a* molecules and a BChl *a* protein with absorption maxima at 808 and 866 nm (B808-866) that is intrinsic to the membrane (Betti et al., 1982; Feick & Fuller, 1984; van Dorssen et al., 1986a). The near-infrared absorption of BChl *c* in chlorosomes is at 740 nm (BChl c_{740}) while that of BChl *a* is at 792 nm (BChl a_{792}). Thus, the energy levels of the *Chloroflexus* antenna pigments are well spaced for the following proposed energy-transfer sequence:

$$(\text{BChl } c_{740} \rightarrow \text{BChl } a_{792})_{\text{chlorosome}} \rightarrow (\text{B808-866} \rightarrow P_{870})_{\text{membrane}}$$

where P_{870}, the reaction center BChl *a*, acts as the terminal energy acceptor. In accordance with its position in this energy-transfer sequence, it has been suggested that BChl a_{792} is located in the chlorosome baseplate (the surface through which the chlorosome is attached to the cytoplasmic membrane) (Feick & Fuller, 1984).

Linear dichroism measurements on oriented *Chloroflexus* chlorosomes have shown that BChl c_{740} is regularly arranged, with its Q_y electronic transition within 40° of being parallel to the long axis of the chlorosome (Betti et al., 1982; van Dorssen et al., 1986a). Electron microscopy of freeze-fractured chlorosomes shows internal rodlike structures that run the length of the chlorosomes (Staehelin et al., 1978; Sprague et al., 1981). These appear to be aggregates of a protein (M_r 5600) that binds and orients BChl *c* (Feick & Fuller, 1984; Wechsler et al., 1985; Brune et al., 1987).

Chlorosomes from *Chlorobium limicola*, a green sulfur bacterium, are structurally similar (Olson, 1980). They also contain internal rod elements running the length of the chlorosome (Staehelin et al., 1980) with associated BChl *c* mol-

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ecules oriented so that their Q_y electronic transitions are parallel to the long axis of the chlorosome (Fetisova et al., 1986; van Dorssen et al., 1986b). *Chlorobium* chlorosomes also contain specialized BChl *a* molecules absorbing at 794 nm (BChl a_{794}) that are thought to mediate energy transfer out of the chlorosome (Gerola & Olson, 1986; van Dorssen et al., 1986b).

In spite of the detailed structural studies on *Chloroflexus* chlorosomes, their operation as energy-collecting devices has been only slightly explored. Some information about energy transfer in *Chlorobium* chlorosomes was obtained by Fetisova and Borisov (1980), who measured fluorescence of BChl *c* in a preparation of photosynthetic membranes with attached chlorosomes using phase fluorometry. From the data obtained, they calculated that 90% of the fluorescence from BChl *c* decayed with a lifetime of 20–60 ps, corresponding to the rate of energy transfer out of the pool of BChl *c* molecules in *Chlorobium* chlorosomes. Fluorescence lifetimes have not been previously reported for *Chloroflexus* chlorosomes.

A standard method for investigating energy transfer in antenna systems is fluorescence lifetime measurement, following a picosecond laser excitation pulse, with single photon counting techniques. This method has been applied successfully to the study of energy transfer in phycobilisomes (Holzwarth, 1986), membrane-attached antenna structures found in cyanobacteria, red algae, and cryptophytes, which are functionally but not structurally analogous to chlorosomes. As in chlorosomes, light absorption in a phycobilisome is followed by energy transfer through a series of pigments with increasingly red-shifted absorption maxima. This eventually produces the excited singlet state of a pigment at the site of phycobilisome attachment to the photosynthetic membrane. In membrane-attached phycobilisomes, the fluorescence lifetime of this pigment is about 100 ps, which corresponds to the rate at which it transfers energy to chlorophyll (Chl) *a* in the photosynthetic membrane. Detaching phycobilisomes from photosynthetic membranes prevents this energy transfer to Chl *a* and increases the lifetime of fluorescence from the pigment at the attachment site to several nanoseconds (Porter et al., 1978; Searle et al., 1978).

The purpose of the work presented here was to determine the lifetimes of the fluorescence from BChl c_{740} and BChl a_{792} in chlorosomes and from BChl a_{866} in membranes from *Chloroflexus*. These lifetimes should be a measure of the rates of energy transfer from BChl c_{740} to BChl a_{792} and from BChl a_{792} to B808-866. In purified, detached chlorosomes, BChl a_{792} should be the terminal acceptor of absorbed light energy, resulting in a substantial increase in its fluorescence lifetime. The measured lifetimes do not entirely support this simple model. The efficiency of energy transfer from chlorosomes to membranes was also determined by comparing excitation spectra for fluorescence from the BChl a_{866} chromophore of B808-866 with absorption spectra of intact cells and of membrane preparations with attached chlorosomes.

Materials and Methods

Chloroflexus aurantiacus strain J-10-fl was grown photoheterotrophically as described previously (Betti et al., 1982). Chlorosomes and membranes with attached chlorosomes (whole membranes) were prepared as described by Feick and Fuller (1984).

Absorption and fully corrected, steady-state fluorescence excitation and emission spectra were measured on an instrument of local design. Light from a 250-W tungsten–halogen lamp was focused onto the entrance slit of a Spex Industries Model 1681 0.22-m single-grating monochromator and then collimated with lenses. A Corning 2-63 filter was inserted at wavelengths greater than 650 nm to reject second-order light. A beam splitter directed about 10% of the monochromatic light to a United Detector Technology (UDT) UDT-220-SB Si photodiode reference detector. The wavelength sensitivity of this detector was determined by comparing its output to the actual intensity of the exciting light as measured by a Molectron Model PR-200 wattmeter. Corrected excitation spectra were then obtained by use of the output of the reference detector corrected for its wavelength response.

The balance of the exciting light was chopped at 225 Hz by a Stanford Research Systems (SRS) chopper Model SR-540 and focused onto the sample. The emitted light was focused onto the entrance slit of a Spex Industries Model 1680 0.22-m double-grating monochromator. A Corning 2-64 filter was inserted before the emission monochromator at wavelengths greater than 700 nm to reject second-order light. A UDT 555D Si photodiode/amplifier detected the emitted light. The diode output was demodulated by an SRS SR-510 lock-in amplifier. An AT&T 6300 personal computer controlled the monochromator stepper motor drives and the lock-in amplifier and collected, stored, analyzed, and plotted the data on a Hewlett-Packard Model 7470 digital plotter. Correction factors for the wavelength dependence of the emission optics, monochromator, and photodiode were generated by calibration with an Optronics 220C NBS-traceable standard tungsten lamp. Excitation and emission corrections were utilized in all spectra.

Absorption spectra were measured in the same instrument using a Labsphere IS060-WR 6-in. diameter integrating sphere with a UDT PIN-10DP/SB Si photodiode detector, with a buffer sample used as a reference in an independent scan.

Fluorescence lifetimes were measured with two separate instruments. The instrument at Simon Fraser University (SFU) has been described previously (Bruce et al., 1985) and produced 0.5-nJ excitation pulses at 725 nm at a frequency of 4 MHz. The pulse duration was 10 ps. A Hamamatsu R1294U-01 microchannel plate photomultiplier was used for measurements at 760 nm and a Varian VPM159A3 photomultiplier, with near-infrared sensitivity extending to 1200 nm, for measurements at 816 and at 894 nm. The emission wavelength was selected with a Spex Industries 0.75-m monochromator. Samples (absorbance at 740 nm = 0.5) were placed in a 100-μL capillary tube (diameter <1 mm) and cooled to 77 K. Fluorescence decay curves were obtained by single photon counting and deconvoluted into up to three components.

The instrument at the Center for Fast Kinetics Research (CFKR) utilized a Spectra Physics 574B dye laser pumped by a Spectra Physics 3000 Nd:YAG laser to provide 727-nm (or 700 nm in a few experiments), 12-ps exciting pulses with an energy of 2.5 nJ and a frequency of 800 kHz. Samples (absorbance at 740 nm = 0.2) were placed in 1-cm square fluorescence cuvettes and measurements made at room temperature. Either a Hamamatsu R928 photomultiplier or, in a few experiments, a Hamamatsu R1294U-01 microchannel plate photomultiplier was used as the detector. The fluorescence emission wavelength was selected with 10-nm band-pass Ditric Optics 3 cavity interference filters, a 760-nm filter for BChl c_{740} emission and either an 800- or an 810-nm filter for BChl a_{792} emission being used. Fluorescence decay curves obtained by single photon counting were deconvoluted into one or two exponentials.

Time-resolved fluorescence emission spectra were obtained with the instrument at SFU by summing the counts over

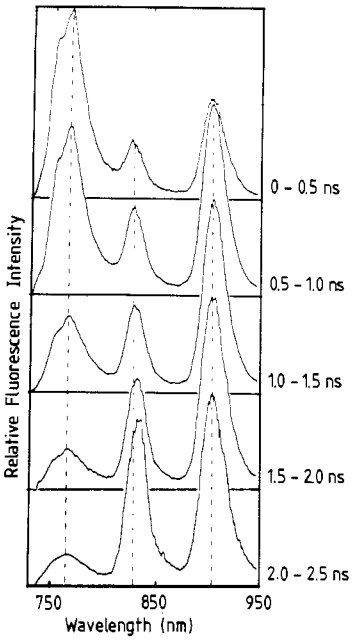

FIGURE 1: Time-resolved 77 K fluorescence emission spectra of *Chloroflexus* membrane fragments. The exciting wavelength was 725 nm. The curves have been normalized to the peak emission.

different time windows following each laser pulse as described by Bruce et al. (1985). These measurements were taken at 1-nm intervals and used the Varian VPM159A3 photomultiplier.

RESULTS

Figure 1 shows a set of fluorescence emission spectra taken on a sample of membranes with attached chlorosomes at the indicated time intervals. Cooling to 77 K caused a bathochromic shift of the emission maxima, so that peaks attributed to BChl c_{740}, BChl a_{792}, and BChl a_{866} occurred at 758, 816, and 894 nm, respectively. The decay rates of the different fluorescence emission peaks depend on the instrument response function as well as on the singlet lifetimes of the fluorescing pigments. Furthermore, the 0.5-ns time window over which counts were collected for each spectrum is long compared to the fluorescence lifetimes of the antenna pigments (see below). Both factors distort the time resolution of these spectra to longer values, and it is not possible to infer excitation transfer kinetics from them. However, they do provide a qualitative picture of excitation transfer. The initial spectrum is dominated by 758-nm emission, which decays rapidly as energy is transferred to the BChl *a* pigments. Emission from these pigments decays more slowly and dominates spectra taken at later time intervals. It is not clear from these spectra whether the rise of 816-nm fluorescence precedes, follows, or is simultaneous with that at 894 nm.

Representative fluorescence decay curves obtained with the two instruments are shown in Figure 2, and a complete summary of the fluorescence lifetimes of the different components of the *Chloroflexus* antenna is presented in Table I. Longer lifetimes for 760-nm fluorescence (from BChl c_{740}) were obtained with the instrument at the CFKR (cf. spectra a and c of Figure 2). This is probably due both to the longer path length of the sample cell (a standard 1-cm square fluorescence cuvette) and to the use of a slower detector in most of these measurements. Excitation is spread over the 30-ps time interval that it takes for light to travel 1 cm, making it difficult to resolve shorter lifetimes. Inherent variability in the transit time for movement of photoelectron pulses through a standard photomultiplier tube such as the R928 further decreased the

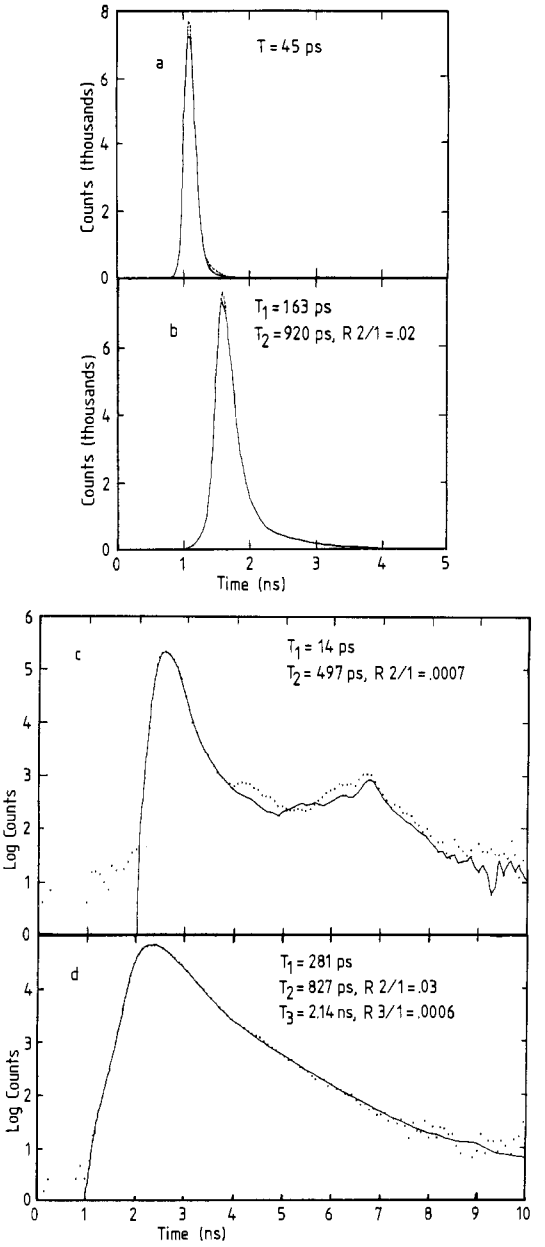

FIGURE 2: Decay curves for fluorescence from chlorosome and membrane pigments. (a and b) Experimental and simulated traces for 760-nm (a) and for 800-nm (b) fluorescence obtained from chlorosomes at the CFKR. The experimental traces are shown as dashed lines and the overlying simulations as solid lines. The indicated lifetimes were used together with the instrument response function to calculate the simulated curves. (c and d) Experimental and simulated traces for 760-nm (c) and for 894-nm (d) fluorescence obtained from membranes with attached chlorosomes at SFU. Note that the results in (c) and (d) are presented in semilog plots. Experimental points are shown as dots, and the trace calculated from the deconvoluted lifetimes and the instrument response function is shown as a solid line. The lifetimes of the first (T_1) and, where measured, the second (T_2) and third (T_3) exponential components of fluorescence decay as well as the ratio of the amplitude of the second to the first [$R(2/1)$] and of the third to the first [$R(3/1)$] component are also indicated.

temporal resolution of these measurements. Thus, the ~50 ± 20 ps lifetime for 760-nm fluorescence obtained at 298 K is more properly regarded as an upper limit than an actual value. Two measurements on isolated chlorosomes in which the R928 detector was replaced with a R1294U-01 microchannel plate photomultiplier, which gives both a shorter and a less variable transit time for photoelectrons, both gave lifetimes (28 and 45 ps) shorter than the 51-ps average obtained with the R928 photomultiplier, supporting the sug-

Table I: Fluorescence Lifetimes for Pigments in Chlorosomes and Membranes from *Chloroflexus*[a]

sample	wavelength (nm)	fluorescence lifetime at 298 K	fluorescence lifetime at 77 K
chlorosomes	760	<50 ps[c,g]	<30 ps[h,m]
	800–816		
	T_1	155 ± 22 ps[f]	200 ps
	T_2	867 ± 238 ps[e]	490 ps
	$R(2/1)$	0.04 ± 0.03[e]	0.13
$K_3Fe(CN)_6$-treated chlorosomes	760	<30 ps[e,i,m,n]	
chlorosomes + 0.67% hexanol	760		
	T_1	75 ps	
	T_2	1060 ps	
	$R(2/1)$	0.01	
membranes	760		
	T_1	<50 ps[b,j]	<30 ps[b,k,m]
	T_2		330 ± 158 ps[b,m]
	$R(2/1)$		0.0005 ± 0.0002
	800–816		
	T_1	93 ± 21 ps[c]	288 ± 56 ps[d]
	T_2	559 ± 23 ps[c]	978 ± 59 ps[d]
	$R(2/1)$	0.09 ± 0.05[c]	0.04 ± 0.06[d]
	894		
	T_1		218 ± 44 ps[c]
	T_2		978 ± 281 ps[c]
	$R(2/1)$		0.008 ± 0.01[c]
membranes + $Na_2S_2O_4$	760	<50 ps[l]	
	800–816		
	T_1	129 ± 16 ps[b]	
	T_2	709 ± 78 ps[b]	
	$R(2/1)$	0.20 ± 0.01[b]	

[a] Samples were suspended in anaerobic 10 mM Tris-HCl, pH 8.0, to give an absorbance of about 0.2 at 740 nm. T_1 and T_2 are the lifetimes of the first and second components of fluorescence emission and $R(2/1)$ is the ratio of the amplitude of the second component to that of the first when more than one component was observed. The third component, obtained in some deconvolutions of 816- and 894-nm fluorescence at 77 K, had a lifetime similar to that of the second component and an amplitude that was always less than 0.2% of that of the first component. Therefore, it is not included here. The excitation wavelength was 725 or 727 nm unless otherwise noted. [b-f] Average and standard deviation from three (*b*), four (*c*), five (*d*), six (*e*), or eight (*f*) determinations. [g-l] Measurements of 760-nm fluorescence lifetimes were instrument-limited and could only establish the upper limits indicated here (see text). The deconvoluted lifetimes (which should not be interpreted as actual lifetimes) were 58 ± 18 (*g*), 4 (*h*), 30 ± 18 (*i*), 48 ± 23 (*j*), 6 ± 7 (*k*), and 47 ps (*l*). [m] Microchannel plate detector. [n] Excitation wavelength was 700 nm.

gestion that the actual lifetime is less than this value. Both the shorter path length of the 100-μL capillary tube used as a sample cuvette and the routine use of the microchannel plate detector to measure 760-nm fluorescence at SFU contribute to the shorter lifetimes obtained with that instrument. This instrument is able to resolve fluorescence lifetimes as short as 30 ps accurately, but it is clear that the values of 4 ps and 6 ± 7 ps obtained by deconvolution are beyond this limit and should in no way be considered to be actual lifetimes. The only conclusion that can be drawn from these results is that the actual lifetime of 760-nm fluorescence is too short to measure with either instrument, being less than 30 ps at 77 K and probably also at 298 K.[1]

The extremely rapid decay of BChl c_{740} fluorescence is probably due to fast excitation transfer to BChl a_{792}. Within the time resolution of the measurements, the lifetime of the 760-nm fluorescence in *Chloroflexus* chlorosomes did not depend on whether or not the chlorosomes were membrane-attached or free in solution. This is consistent with the suggestion that the BChl *c* excited state is quenched by rapid excitation transfer to BChl a_{792} within the chlorosome.

Attempts to increase the lifetime of 760-nm fluorescence by disrupting excitation transfer to BChl a_{792} had no obvious effect. In one of these, adding hexanol selectively disrupted part of the BChl c_{740} chromophore to a presumably monomeric form of BChl *c* absorbing maximally at 670 nm (Brune et al., 1987). A hexanol concentration of 0.67% produced a chlorosome suspension with equal absorbances at 740 and 670 nm. The lifetime of 760-nm fluorescence from this sample was 75 ps (Table I). Although this is longer than the 50-ps average deconvoluted lifetime, it is not outside the range of values found with untreated chlorosomes. Further measurements are needed to determine whether this increase in lifetime is significant. A second component, with a lifetime of 1.06 ns, that accounted for about 1% of the initial amplitude of 760-nm fluorescence probably comes from a small amount of dissociated BChl *c* that was excited by the 727-nm laser pulses.

In another procedure, chlorosomes were treated with an excess of $K_3Fe(CN)_6$, which was then removed by repeated cycles of concentration in a Centracon 30 ultrafiltration unit (Amicon Corp.) followed by dilution with fresh buffer. This caused a substantial decrease in absorbance at 792 nm relative to that at 740 nm and decreased the amount of steady-state fluorescence at 800 nm relative to that at 750 nm by about 45% (data not shown). Nevertheless, fluorescence lifetimes measured with the microchannel plate photomultiplier averaged 30 ± 18 ps, which is not significantly different from the results obtained with untreated chlorosomes by the same detector.

Fluorescence decay at 800–816 nm (from BChl a_{792}) was analyzed as two exponentials. Most (usually more than 90%) of the fluorescence decayed rapidly, having a measured lifetime of 155 ± 22 ps in isolated chlorosomes and 93 ± 21 ps in membrane-bound chlorosomes (at 298 K). The approximately 1.7 times faster decay in membrane-bound chlorosomes is probably due to energy transfer from BChl a_{792} in the chlorosomes to B808-866 in the membrane.

From the room temperature data on BChl a_{792} fluorescence, the mean rate constant $(1/T_1)$ for decay of the singlet excited state of BChl a_{792} (BChl a_{792}^*) was calculated to be 6.5×10^9/s in detached chlorosomes and 1.1×10^{10}/s in attached chlorosomes. If one assumes that the rate of deactivation of BChl a_{792}^* by processes other than excitation transfer does not depend on whether or not the chlorosomes are membrane-attached, the rate constant for excitation transfer from BChl a_{792}^* to B808-866 in the membrane is then $(11-6.5) \times 10^9$/s or 4.5×10^9/s. These figures indicate that excitation transfer from the chlorosome to the photosynthetic membrane is no more than 41% efficient.

An independent measurement of the efficiency of excitation transfer obtained by comparing a spectrum for excitation of fluorescence from BChl a_{866} with the absorption spectrum of a whole membrane preparation showed that light absorbed by BChl c_{740} is 60% as efficient at exciting fluorescence from BChl a_{866} at 910 nm as is light absorbed by B808-866 itself (Brune & Blankenship, 1987). (The amount of light absorbed directly by BChl a_{792} is too small for it to be distinguished in either the absorption or fluorescence excitation spectrum.) This implies that BChl a_{792} to B808-866 excitation transfer is at least 60% efficient, and an even higher efficiency must be assumed if excitation transfer from BChl *c* to BChl a_{792} is less than 100% efficient.

[1] Preliminary streak camera measurements performed in collaboration with B. Wittmershaus, S. Lin, and R. S. Knox indicate that the actual lifetime of 760-nm fluorescence is 15 ps in isolated chlorosomes at room temperature.

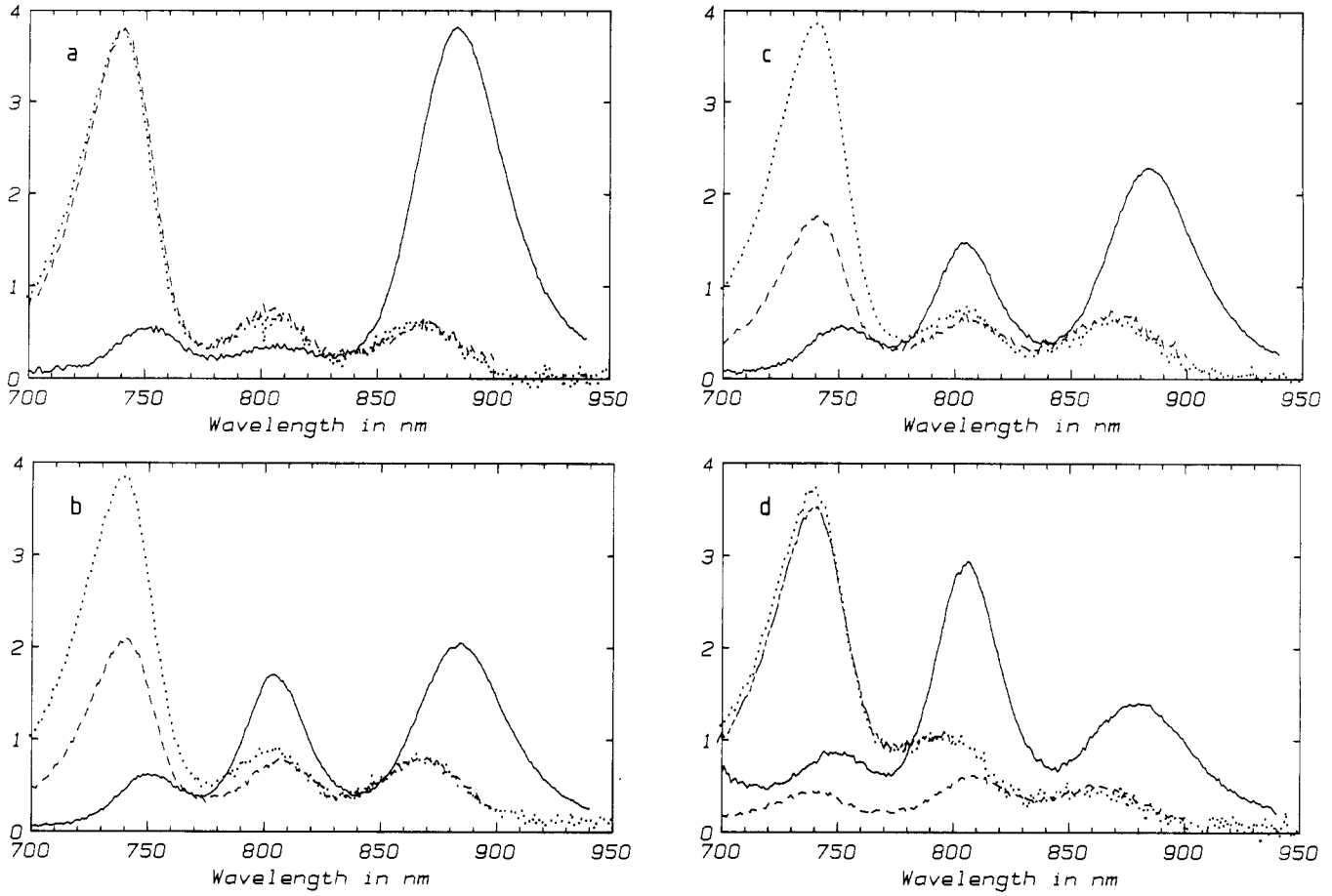

FIGURE 3: Absorption and fluorescence excitation and emission spectra of *Chloroflexus* cells and subcellular preparations. (a) Intact *Chloroflexus* cells suspended in 10 mM Tris-HCl, pH 8, plus 0.15 M NaCl (isotonic buffer). (b) Cells after sonication in 10 mM Tris-HCl, pH 8, containing 2 mM phenylmethanesulfonyl fluoride. (c) Photosynthetic membranes obtained by centrifuging sonicated cells. (d) Membranes plus 0.3% miranol. (···) Absorption spectrum; (--) excitation spectrum for 920-nm fluorescence (14.4-nm spectral bandwidth); (-·--) excitation spectrum for 820-nm fluorescence (14.4-nm spectral bandwidth) in panel d; (—) fluorescence emission spectrum. All absorption spectra were measured with a 1.8-nm spectral bandwidth by use of an integrating sphere to avoid light-scattering artifacts. Fluorescence excitation spectra were obtained by use of a 9-nm spectral bandwidth for the exciting light. The actinic wavelength for fluorescence emission was 460 nm (18-nm spectral bandwidth), and emission was monitored with a spectral bandwidth of 10.8 nm. Absorption and excitation spectra were normalized at 866 nm for 920-nm fluorescence and at 795 nm for 820-nm fluorescence. Fluorescence emission spectra are all plotted on the same scale, corrected for slight differences in sample concentration, which in all cases was adjusted to give an absorbance of about 0.1 at 740 nm.

No difference between the lifetimes of BChl a_{792} fluorescence in attached and detached chlorosomes was observed at 77 K, at which temperature the fluorescence lifetime was slightly longer. This may indicate that the efficiency of excitation transfer from chlorosomes to membranes is lower at 77 K than at room temperature, but independent experiments have not yet been done to test this possibility.

Because our data indicated surprisingly low chlorosome to membrane excitation transfer efficiencies in the membrane preparations used for fluorescence lifetime measurements, excitation transfer efficiencies in intact cells and after different treatments used in membrane and chlorosome isolation were also determined (Figure 3). Fluorescence excitation and absorption spectra of intact *C. aurantiacus* cells were superimposable (Figure 3a), indicating (within the limits of accuracy of these measurements) a 100% efficiency of excitation transfer from BChl c_{740} in the chlorosomes to BChl a_{866} in the membrane. The fluorescence emission spectrum also indicates efficient energy transfer. Most of the emission was in a peak centered at 885 nm, with weaker maxima at 750 and 804 nm, in spite of the fact that the excitation wavelength (460 nm) was absorbed preferentially by BChl c_{740}.

The ability of chlorosomes to transfer energy to the photosynthetic membrane was found to be quite sensitive to treatments used during membrane isolation. Sonicating the cells caused a 45% decrease in efficiency, as measured by the decreased height of the 740-nm peak in the excitation, relative to the absorption, spectrum (Figure 3b). This decreased efficiency of excitation transfer was accompanied by increased emission at 804 nm and decreased 885-nm emission, suggesting that energy transfer is partially blocked at the BChl $a_{792} \rightarrow$ B808-866 step. Freezing *C. aurantiacus* cells produced similar effects, also causing an approximately 45% decrease in the excitation transfer efficiency accompanied by increased emission at 804 nm (not shown).

Membranes with attached chlorosomes, prepared by differential centrifugation of sonicated cells, exhibited a 50% excitation transfer efficiency and generally had fluorescence excitation and emission spectra similar to those of sonicated cells (Figure 3c). The excitation transfer efficiency was further decreased by freezing and thawing the membranes (not shown). There is clearly some variability in different preparations. For example, one of the membrane preparations used for fluorescence lifetime measurements exhibited a 60% energy transfer efficiency even after freezing and thawing, as reported earlier (Brune & Blankenship, 1987). Attempts to separate chlorosomes from membranes on a sucrose density gradient gave only a single band, having the absorption spectrum of membranes with attached chlorosomes. Fluorescence excitation, emission, and absorption spectra of samples taken from

different levels within this band were identical (not shown), indicating that the decreased excitation transfer efficiency is not due to physical detachment of chlorosomes from the membranes.

Adding 0.3% miranol to the membrane preparation, which physically detaches the chlorosomes and allows them to be separated on a sucrose density gradient, decreased the efficiency of excitation transfer to 12% in the experiment shown in Figure 3d and caused the intensity of emission at 804 nm to exceed that at 885 nm. Comparison of the absorption spectrum with an excitation spectrum obtained between 700 and 800 nm for the 804-nm fluorescence (measured at 820 nm) indicated a 91% efficiency of excitation transfer from BChl c_{740} to the chromophore (presumably BChl a_{792}) responsible for this fluorescence. Interestingly, the intensity of fluorescence emission at 804 nm in our purified chlorosomes was only 1.1–1.6 times that of the 750-nm emission (not shown), and the emission intensities are about equal in published spectra (Betti et al., 1982; van Dorssen et al., 1986a), while in Figure 3d the intensity of emission at 804 nm exceeded that at 750 nm by a factor of 3.4. This suggests that additional processes for quenching 804-nm fluorescence arise during later steps in the purification of detached chlorosomes.

A small portion (0.1–14%) of the 800–816-nm fluorescence decayed with a lifetime of 0.5–1.3 ns (Table I). Neither the amplitude nor the lifetime of this long-lived component show any clear correlation with attachment or detachment of chlorosomes and membranes, suggesting that the pigment from which it arises does not participate in energy transfer in the *Chloroflexus* antenna.

Fluorescence at 894 nm from the membrane antenna protein B808-866 was measured at 77 K. A total of 97–99% of the fluorescence from this component decayed with a lifetime of 218 ± 44 ps. This is in good agreement with the 200-ps lifetime found at room temperature by Nuijs et al. (1986) for the excited singlet state of this antenna pigment in purified membranes with P_{870} oxidized from *Chloroflexus*. Similar excited singlet lifetimes have been observed for the functionally analogous pigments B875 in *Rhodobacter sphaeroides* and B880 in *Rhodospirillum rubrum* in chromatophores with oxidized reaction centers (Borisov et al., 1985; Sundström et al., 1986).

Discussion

Fluorescence lifetime measurements demonstrate that excitation transfer out of the BChl c_{740} component of the antenna in chlorosomes is extremely rapid, probably occurring in less than 30 ps. These results are consistent with the 20–60-ps lifetime of BChl *c* fluorescence found in *Chlorobium* chlorosomes, which was also at the instrumental limit of resolution, by phase fluorometry (Fetisova & Borisov, 1980). In both *Chloroflexus* and *Chlorobium* chlorosomes, BChl *c* occurs in a highly ordered array [for further discussion, see Brune et al. (1987)]. It is likely that this orderly arrangement is responsible for fast energy transfer out of the pool of approximately 10000 BChl *c* molecules in the chlorosome. Fetisova et al. (1986) have previously discussed the effects of various possible BChl *c* orientations on the rate of energy transfer from *Chlorobium* chlorosomes, although their discussion did not explicitly consider a role for BChl a_{794} (the *Chlorobium* pigment analogous to BChl c_{792} in *Chloroflexus*), which was discovered only recently in *Chlorobium* chlorosomes (Gerola & Olson, 1986; van Dorssen et al., 1986b).

The lifetime of fluorescence at 800–810 nm (from BChl a_{792}) was surprisingly insensitive to whether or not the chlorosomes were membrane-attached, being only about 1.7 times higher in detached than in attached chlorosomes. This is completely different from the situation with phycobilisomes, in which detachment from the membrane increases the fluorescence lifetime of the pigment mediating excitation transfer into the membrane from about 100 ps to several nanoseconds (Porter et al., 1978; Searle et al., 1978). These results are, however, consistent with our observation that steady-state fluorescence at 800 nm was only slightly higher, relative to 755-nm fluorescence, in detached than in membrane-bound chlorosomes (by a factor of 1.6 at most in the preparations used for fluorescence lifetime measurements).

Comparison of the absorption spectrum with the fluorescence excitation spectrum of a *Chloroflexus* membrane preparation used for fluorescence lifetime measurements indicated a chlorosome-to-membrane excitation transfer efficiency of 60% (Brune & Blankenship, 1987). Although this value is higher than the 41% calculated from fluorescence lifetimes, it is still quite low. It is more difficult to reconcile the 800–816-nm fluorescence lifetime data with the essentially 100% efficiency of excitation transfer found in intact cells. Assuming that the measured value of 155 ps is the lifetime of BChl a^*_{792} in chlorosomes when energy transfer is prevented without affecting other deactivation process, this lifetime would have to decrease to less than 15 ps in order to obtain an excitation transfer efficiency higher than 90%.

Both the measured 155-ps lifetime of 800–810-nm fluorescence from detached chlorosomes and the <15-ps lifetime postulated in intact cells are unusually short compared to excited singlet state lifetimes of BChl *a* in antenna complexes of other photosynthetic bacteria. For example, the lifetime of BChl a^*_{850} in the B800-850 complex from *Rhodobacter sphaeroides* is about 1 ns in the purified complex (Sebban et al., 1984; Bergström et al., 1986). Time-resolved absorbance measurements on B800-850 in *R. sphaeroides* chromatophores indicate a 60-ps lifetime for excitation transfer to B875 in the intact membrane (Sundström et al., 1986). Similarly, the lifetime of fluorescence from BChl a_{875} in the B875 complex is about 650 ps (Sebban et al., 1985) when energy transfer to the reaction center is prevented either by isolation and purification of B875 or by elimination of the reaction centers through mutation. Energy transfer to photochemically active reaction centers decreases this lifetime to 60 ps in wild-type bacterial chromatophores (Borisov et al., 1985). [The close spacing of the energy levels of BChl a_{850} and BChl a_{875} in the B800-850 and B875 complexes results in an equilibrium distribution of excited states between these chromophores and somewhat complicates the overall pattern of excitation transfer in *R. sphaeroides* (Sundström et al., 1986), but this should not be a factor in *C. aurantiacus* where the antenna energy levels are more widely separated.] On the basis of these comparisons, the expected lifetime of BChl a^*_{792} in isolated chlorosomes should be closer to the 500–1000-ps value found for the minor component than to the 155-ps lifetime of the major component of fluorescence emission.

A possible explanation for the surprisingly short lifetime of 800–810-nm fluorescence from isolated chlorosomes might be that isolation causes alterations in the environment of BChl a_{792} that accelerate decay of its excited state. Alternatively, the alteration decreasing the BChl a_{792} excited-state lifetime might be chemical. Bergström et al. (1986) noted that the lifetime of BChl a^*_{850} in the isolated B800-850 complex from *R. sphaeroides* decreases from 0.9 ns to 70–100 ps in the presence of oxygen and proposed that BChl a^*_{850} was quenched by photochemically formed BChl *a* oxidation products. Although our fluorescence lifetime measurements were performed

on samples in degassed buffer in cuvettes stoppered under N_2, chlorosomes and photosynthetic membranes were prepared under aerobic conditions, and it is possible that BChl oxidation products accumulated during the preparation. If so, the 155-ps (298 K) or 200-ps (77 K) major component of 800–816-nm fluorescence would be the lifetime of BChl a_{792} fluorescence quenched by oxidation products while the 500–1000-ps minor component might arise from intact BChl a_{792}. Partial re-reduction of these oxidation products may explain the slight increase in the relative amplitude of the longer lived component when $Na_2S_2O_4$ was added to a photosynthetic membrane preparation (Table I), although closing reaction centers in the membrane by reducing the primary electron acceptor is an alternative explanation. Generation of stable and potent quenchers by BChl *a* oxidation could also explain why preferential destruction of BChl a_{792} by $K_3Fe(CN)_6$ failed to increase the lifetime of fluorescence from BChl c_{740}. This interpretation implies that BChl a_{792} is unusually sensitive to oxidation, since no need to maintain anoxic conditions during preparation of B800-850 has been noted. Preliminary experiments indicate that use of anaerobic buffers yields isolated chlorosomes with higher steady-state fluorescence at 805 nm, thus supporting the suggestion that oxidizing conditions create quenchers.

In summary, while our data show a low chlorosome-to-membrane excitation transfer efficiency in standard whole membrane preparations from *Chloroflexus*, this is not the case in fresh, intact cells. Additional studies are needed to examine some of the suggested explanations for this low efficiency and to obtain membrane preparations with a higher efficiency. Use of anaerobic buffers and variations in the ionic strength and temperature during membrane isolation might yield preparations with more efficient chlorosome to membrane excitation transfer. Energy transfer from BChl c_{740} to BChl a_{792} in both free and membrane-bound chlorosomes should be investigated with instruments able to resolve fluorescence lifetimes less than 30 ps. These measurements might explain the decrease in the efficiency of energy transfer from BChl c_{740} to BChl a_{792} that has been reported to occur during chlorosome isolation (van Dorssen et al., 1986a). Finally, fluorescence lifetime measurements on BChl a_{794} in *Chlorobium* chlorosomes might be revealing. Although *Chlorobium* chlorosomes are structurally similar to those from *Chloroflexus*, their mode of attachment to membranes and the procedure for isolating them are different (Gerola & Olson, 1986). Thus, the lifetime of fluorescence from BChl a_{794} in isolated chlorosomes might not be anomalously short, as appears to be the case with the fluorescence of BChl a_{792} in *Chloroflexus* chlorosomes.

Added in Proof

More recent measurements (Wittmershaus et al., 1988) indicate that the efficiency of excitation transfer from BChl c_{740} to BChl a_{866} in whole cells under physiological conditions (55 °C) with weak excitation intensity is 69 ± 13%. We believe that the somewhat higher efficiency observed here at room temperature (100%) is partially due to actinic effects of the excitation beam, which are greater at 740 nm than at 866 nm.

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