Excitation delocalization in the bacteriochlorophyll *c* antenna of the green bacterium *Chloroflexus aurantiacus* as revealed by ultrafast pump-probe spectroscopy

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Abstract Room temperature absorption difference spectra were measured on the femtosecond through picosecond time scales for chlorosomes isolated from the green bacterium *Chloroflexus aurantiacus*. Anomalously high values of photoinduced absorption changes were revealed in the BChl *c* Q_y transition band. Photoinduced absorption changes at the bleaching peak in the BChl *c* band were found to be 7–8 times greater than those at the bleaching peak in the BChl *a* band of the chlorosome. This appears to be the first direct experimental proof of excitation delocalization over many BChl *c* antenna molecules in the chlorosome.

Key words: Bacterial photosynthesis; Antenna pigment organization; Bacteriochlorophyll *c*; Green photosynthetic bacteria; Ultrafast spectroscopy; Absorption difference spectroscopy

1. Introduction

Our recent works have focused on the development of adequate models for both the three-dimensional and electronic structure and function of the largest among all known photosynthetic antennae – the chlorosome of green bacteria [1–12]. The chlorosome of Chloroflexaceae green bacteria typically contains several thousand bacteriochlorophyll (BChl) *c* pigments absorbing at ~740 nm, which appear to be organized in the form of hollow cylinders (rod elements) each ~5 nm in diameter and ~100 nm long [13]. Electronic excitations in the oligomeric, light-harvesting 740 nm BChl *c* antenna travel to a 795 nm BChl *a* chlorosome baseplate antenna with multiexponential kinetics [5,6] dominated by a 10–40 ps component whose lifetime depends on the BChl *c* antenna size [9]. Plausible models of BChl *c*/*d*/*e* aggregates (for review see [1,9,11]) have been recently examined by using the exciton level structure criterion [9,11]. It was shown that none of the hitherto proposed molecular models of BChl *c*/*d*/*e* aggregation in the chlorosome displays the in vivo exciton level structure of a BChl aggregate, revealed by spectral hole burning experiments [2–4]. A new original exciton model of aggregation of chlorosomal pigments BChl *c*/*d*/*e* has been proposed [9,11]. This model of six linear (single or double) exciton-coupled BChl chains, arranged in a tubular shape, with a low packing density, approximating that in vivo, and interchain distances of ~2 nm, was generated to yield the key spectral features found in natural antennae, i.e. the exciton level structure with strongly allowed higher levels [2–4], and the polarization of all the levels parallel to the long axis of the chlorosome [3–15]. The theory of excitation energy transfer within an oligomeric type light-harvesting antenna has been developed for the first time, and, in particular, within the chlorosome of green bacteria [9]. With picosecond fluorescence spectroscopy, it was demonstrated that this newly developed theory explains antenna-size-dependent exciton dynamics in the chlorosomal antenna, measured for intact cells of different cultures of the green bacterium *Chloroflexus aurantiacus* with different chlorosomal antenna sizes determined by electron microscopic examination of ultrathin sections of the cells [9,10]. Analogous data were obtained for the BChl *c* antenna in the chlorosome of the green bacterium *Chlorobium limicola* [12].

Thus, our model of structure and function of the chlorosome implies that Q_y electronic excitation is delocalized over a tubular BChl *c*/*d*/*e* aggregate. So, the detailed structure of a BChl aggregate should be examined by the direct measurement of the extent of excitation delocalization [16]. The direct method for determination of the effective exciton domain size in an aggregate is non-linear (such as pump-probe) spectroscopy. It is known that the non-linear response of a molecular aggregate is very sensitive to the aggregate size (i.e. the number of strongly coupled molecules) in a manner that depends on the pigment organization within an aggregate [16–18].

In this work, with pump-probe spectroscopy under femtosecond resolution, anomalously large induced absorption in the BChl *c* antenna of the green bacterium *Cf. aurantiacus* was demonstrated for the first time, providing direct evidence of exciton delocalization over a BChl *c* aggregate.

A theoretical consideration of the data based on exciton models will be deferred to a later article (work in progress).

2. Materials and methods

All experiments were performed on chlorosomes isolated from the thermophilic green bacterium *Cf. aurantiacus* (strain Ok-70-fl) by standard methods [19] with slight modifications [14]. In all experiments, we used chlorosomes containing only one layer of rods. To this end, each *Cf. aurantiacus* cell culture, grown under high light conditions, was examined with electron microscopy [9]. Electron microscopic observations were made with a Hitachi-12 electron microscope operating at 75 kV. The cells were fixed in the culture medium

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Abbreviations: BChl, bacteriochlorophyll; CMC, chlorosome-membrane complex; ESA, excited state absorption; SE, stimulated emission; PB, photobleaching

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at 55°C with glutaraldehyde, postfixed with OsO_4, embedded in Epon-812, and ultrathin sectioned by standard methods [20]. Micrographs of the ultrathin sections were used for morphometric measurements (magnification 50 000×10) to obtain histograms of chlorosome heights and to calculate the number of layers of rod elements in a chlorosome, using the results reported by Sprague et al. [21].

The self-mode-locked Ti:sapphire laser and pump-probe optics have been described elsewhere [5,6,22]. In one-color experiments, the laser output was tuned with a single-plate birefringent filter, producing ~80 fs pulse width and ~10 nm spectral bandwidth. In two-color experiments, the birefringent filter was omitted, yielding ~40 fs pulses and ~20–40 nm bandwidth. The pump and probe spectra were independently shaped using ~7 nm bandpass interference filters (CVI Corp). The laser cross-correlation in two-color experiments was typically 250–300 fs. Samples under investigation were housed in a high-speed centrifugal sample cell which provided 4 m/s sample travel through the laser beams (sample turnover time is ~6 μs). Excitation pulses generally excited 1 out of every 2000 BChl *c* pigments (average pump power <0.4 mW at ~75 MHz repetition rate); annihilation effects were absent, because ΔA profiles showed no variation with laser power. The apparatus instrument function was recorded concurrently with every pump-probe scan. The laser pump and probe spectra were measured during experiments using a Czerny-Turner monochromator (7.9 nm/mm dispersion).

Chlorosome absorption difference spectra were compiled by amassing two-color pump-probe profiles for a number of probe wavelengths under fixed pump wavelength. While changes in probe wavelength entailed minor optimization in probe beam alignment, the precision in relative amplitudes of the resulting pump-probe profiles was not worse than 5%.

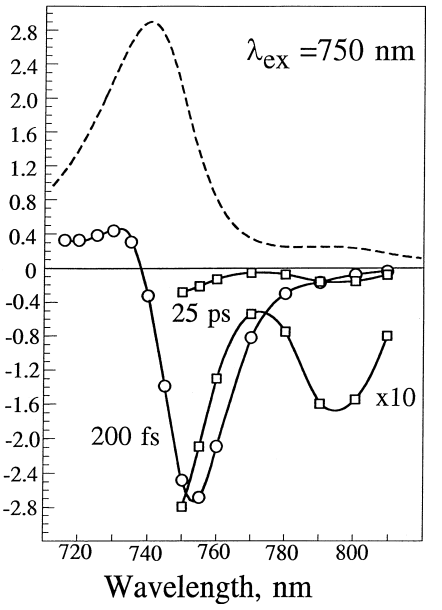

Fig. 2. Absorption difference spectra of chlorosomes at fixed time delays 200 fs (○) and 25 ps (□) after 750 nm excitation, evaluated from the isotropic profiles in Fig. 1. The chlorosome steady-state absorption spectrum is superimposed (dashed curve), showing BChl *c* and BChl *a* band maxima at 740 and 795 nm, respectively.

3. Results and discussion

We investigated excited state kinetics of chlorosomes (and chlorosome-membrane complexes (CMCs)) from *Cf. aurantiacus* under fs resolution.

Earlier we have shown by electron microscopic examination of ultrathin sections of *Cf. aurantiacus* cells that any cell culture can be characterized by its own discrete distribution function *F(p)*, where *F(p)* is the relative number of chlorosomes *F* with *p* rod layers [9]. We discovered that this is a common feature for *Cf. aurantiacus* [9], *Cb. limicola* [12], as well as for *Cb. phaeovibrioides* and *Cb. phaeobacteroides* (not published) cell cultures, regardless of whether these cultures are continuous or batch ones. Thus, in any real object under investigation one can register only the result of a simultaneous functioning of chlorosomal antennas that differ in their sizes and consequently in times of BChl *c*→Bchl *a* excitation energy transfer (for instance, up to four times in one and the same *Cf. aurantiacus* cell culture [9]). Thus, any analysis of experimental exciton dynamics in the chlorosomal antenna must consider this important intrinsic feature of any real object. This fact significantly complicates an analysis of experimental data. In order not to take into account biological disorder of this type, we used chlorosome samples isolated from *Cf. aurantiacus* cells, containing only one rod layer in chlorosomes. This was checked by an analysis of ultrathin sections of cells under investigation [9] before the chlorosome and/or CMC isolation procedure. With electron microscopy, we discovered that in such cells the ratio of absorption of BChl *c* and of BChl *a* at their maxima, A_{740}/A_{866}, is less than 10. This conclusion has been confirmed independently by measuring two-color pump-probe profiles for two different CMCs samples with ratios $A_{740}/A_{866}=4$, and $A_{740}/A_{866}=8$. As should be expected, the samples proved to be kinetically identical (not shown). Besides, ps components of BChl *c*→BChl *a* excitation energy transfer in both samples have been shown to be identical (~11 ps) and to equal those we measured earlier in similar intact cells, having predominantly one layer of rods in chlorosomes (~12 ps) [9].

Chlorosomes were excited at 750 nm at room temperature, and probed at 11 wavelengths from 720 to 809 nm. The B740–795 steady-state absorption spectrum remained essentially unchanged in the region during the course of the experiments. The resulting isotropic absorption difference profiles are shown in Fig. 1. This figure accurately reflects the relative

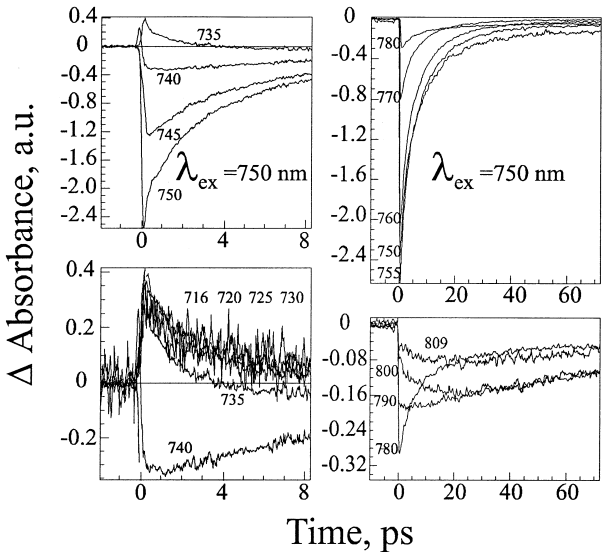

Fig. 1. Isotropic two-color profiles for chlorosomes from *Cf. aurantiacus* excited at 750 nm at room temperature, and probed at several wavelengths from 716 to 809 nm; time windows are 8 ps (left) and 70 ps (right). Signals for different probe wavelengths are mutually normalized; absorbance units are otherwise arbitrary. Positive and negative signals correspond to ESA and PB/SE, respectively.

signal amplitudes for different probe wavelengths: the 750 nm excitation laser conditions were identical for all profiles, and the probe pulse intensities were separately measured with a monitor photodiode for mutual normalization at different probe wavelengths. At probe wavelengths from 750 nm to the red edge of the chlorosome band (Fig. 1, right), all profiles show only photobleaching/stimulated emission (PB/SE) signals. At shorter probe wavelengths (Fig. 1, left), the principal initial feature is an excited state absorption (ESA) component. All kinetics phenomena are analogous to those described in detail in earlier experiments [5,6].

Fig. 2 shows absorption difference spectra at fixed time delays (200 fs and 25 ps) assembled from the profiles in Fig. 1. These ΔA spectra are superimposed on the chlorosome steady-state absorption spectrum, measured in the same rotating absorption cell used in pump-probe experiments. The principal features of these ΔA spectra are similar to those published previously [6]. At 200 fs delay (which overlaps the laser cross-correlation function), the signal exhibits a B740 PB/SE maximum near 752 nm, accompanied by a pronounced B740 ESA maximum near 725 nm. By 25 ps (when $\sim$95% of all excitations transferred from BChl *c* to BChl *a*), the BChl *a* ΔA spectrum is developed. This spectrum (shown in Fig. 2 also on 10-fold-expanded scale) exhibits a B795 nm PB/SE peak. Note that the sigmoid ΔA spectrum of the BChl *c* antenna, with a major ESA peak to the blue of the PB/SE peak, is characteristic of strongly coupled aggregates [6,17]. No analogous ESA feature is found in the BChl *a* ΔA spectrum at wavelengths to the blue of 795 nm. The shape of the BChl *c* ΔA spectrum fully reproduces the shape of the B795 steady-state absorption spectrum, and the position of the absorption maximum (795 nm) is near that for monomeric BChl *a* in vitro. Thus, one can conclude that the B795 pigments are functionally monomeric. Moreover, our pump-probe experiments on CMCs, isolated from the same *Cf. aurantiacus* cell culture, strongly suggest that the entire BChl *a* band with absorption maximum at $\sim$805 nm (which is a superimposition of the B795 and B808 bands) is due to pigments, functioning essentially as monomeric ones [23].

Thus, measurements of ΔA signals in the 740 and 795 nm bands can be used in principle to infer the effective exciton size in the strongly exciton-coupled BChl740 aggregate, i.e.

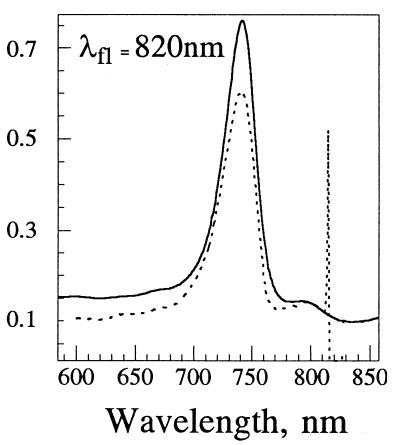

Fig. 3. Chlorosome steady-state absorption (solid curve) and fluorescence excitation (dashed curve) spectra in arbitrary units; fluorescence excitation spectrum was measured at BChl *a* fluorescence wavelength 820 nm.

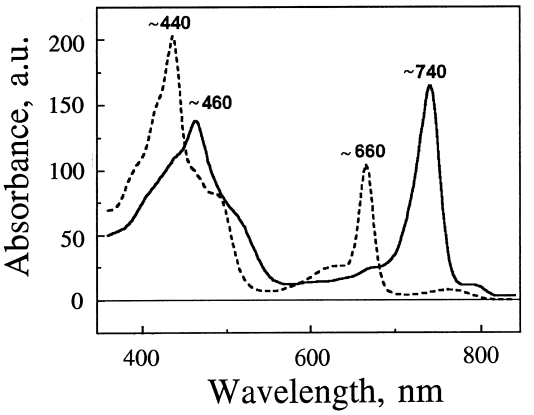

Fig. 4. Absorption spectra of isolated chlorosomes (1) in 10 mM Tris buffer (solid curve), and (2) in acetone-methanol (7/2; v/v) with the same sample concentration as in (1) (dashed curve).

the effective number of BChl *c* molecules over which exciton wavefunctions have sizeable amplitudes [16]. According to Fig. 2, the experimental ratio of maximal PB/SE signals in these two bands (called the bleaching ratio) ΔA_{752} (200 ps)/ΔA_{795} (25 ps) is 16. Note that the bleaching ratio, measured experimentally, gives an upper limit. In order to get the true value of the bleaching ratio, we should take into account the efficiency (ϕ) of BChl *c* $\rightarrow$ BChl *a* excitation energy transfer, and the hyperchromism (H) of the aggregated BChl *c* Q_y band. The Q_y band of an aggregate of n BChl *c* molecules contains $\eta = n \times H$ times the oscillator strength of a single pigment.

The efficiency of BChl *c* $\rightarrow$ BChl *a* excitation energy transfer in the chlorosome was estimated from the steady-state BChl *a* fluorescence excitation spectrum (Fig. 3). The efficiency has been shown to equal $\phi \cong 76\%$. The hyperchromism value for BChl *c* Q_y band of the chlorosome was determined by comparing the oscillator strength values of the near infrared Q_y transition bands (1) for monomeric BChl *c* in vitro (660 nm band), i.e. for chlorosomes under investigation in acetone-methanol, and (2) for BChl *c* in vitro, i.e. for chlorosomes under study with the same material concentration as in (1) (Fig. 4). The hyperchromism value has been shown to be equal to $H = 1.60 \pm 0.05$. Then, the true value of the bleaching ratio ΔA_{752} (200 ps)/ΔA_{795} (25 ps) is $16 \times (0.76/1.6) = 7.6$.

Thus, photoinduced absorption changes at the bleaching peak in the BChl *c* Q_y transition band were found to be several times greater than those at the bleaching peak of the monomeric BChl *a* band of the chlorosome. The data provide the first direct observation of excitation delocalization over many BChl *c* antenna molecules in the chlorosome.

We will show in the following paper that the value of the bleaching ratio obtained considerably limits the range of possible effective BChl *c* exciton sizes and the range of possible sizes of an elementary BChl *c* aggregate in chlorosomes under investigation.

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