Light control over the size of an antenna unit building block as an efficient strategy for light harvesting in photosynthesis

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Abstract **It was shown that an increase in the bacteriochlorophyll (BChl) *c* antenna size observed upon lowering growth light intensities led to enhancement of the hyperchromism of the BChl *c* Q_y absorption band of the green photosynthetic bacterium *Chloroflexus aurantiacus*. With femtosecond difference absorption spectroscopy, it was shown that the amplitude of bleaching of the oligomeric BChl *c* Q_y band (as compared to that for monomeric BChl *a*) increased with increasing BChl *c* content in chlorosomes. This BChl *c* bleaching amplitude was about doubled as the chlorosomal antenna size was about trebled. Both sets of findings clearly show that a unit BChl *c* aggregate in the chlorosomal antenna is variable in size and governed by the grow light intensity, thus ensuring the high efficiency of energy transfer within the BChl *c* antenna regardless of its size. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Light-harvesting photosynthetic antenna; Green bacterium

1. Introduction

Theoretical analysis of the key steps in the primary conversion of light energy in photosynthesis showed that the light-harvesting antenna structure must be highly optimized to operate with an observed 90% quantum yield [1], which means that an antenna is neither uniform nor isotropic. At the same time, a long-range molecular order in natural antennae must be in existence, since only ordered systems may be optimized [1,2]. Some basic principles for designing optimal light-harvesting systems were suggested, and targeted searches for these principles allowed us to recognize some of them in natural antennae [1–5].

Most photosynthetic organisms are able to adapt to low light intensities by increasing drastically the antenna size (i.e. the number of antenna molecules per reaction center) due to peripheral antennae thus making, however, the problem of optimization for variable antennae more acute because larger antennae place even more severe requirements for optimization [1].

We have previously shown that antenna pigment aggregation, being universal to lattices of any geometrical type, is a biologically expedient strategy for light harvesting [2,7,8]. In particular, it was shown that an increase in the unit aggregate size results in an increase in the efficiency of the antenna functioning. Therefore an investigation of possibility of realization of such phenomenon in oligomeric antennae of those photosynthetic organisms, which are able to grow in a wide range of light intensities due to significant changes of the antenna size [6], is of value, as the requirements for structural optimization become more rigorous with increasing the antenna size [1]. The green non-sulfur bacterium *Chloroflexus aurantiacus* was chosen for such studies as a most suitable object, which possesses a peripheral oligomeric antenna called the chlorosome. In this organism, chlorosomes are the principal light-harvesting extramembrane structures containing several thousand molecules of the main light-harvesting pigments bacteriochlorophyll (BChl) *c* which are thought to be organized in rod-like elements. Besides BChl *c*, chlorosomes contain carotenoids and a small amount of BChl *a*. This BChl *a* antenna connects the chlorosomal BChl *c* antenna with the cytoplasmic membrane, in which the core BChl *a* antenna and reaction centers are located [9,10]. This organism (as well as other green bacteria) is able to grow in a wide range of light intensities. As the light intensity decreases, the size of the BChl *c* antenna increases [6] thus enhancing the light-absorbing capability of the antenna. However, the efficiency of the antenna functioning must inevitably drop with increasing its size [1]. In this work, we show that the size of a unit BChl *c* aggregate in the *C. aurantiacus* chlorosome is light-controlled, thus promoting the high efficiency of energy transfer within the BChl *c* antenna regardless of its size.

2. Materials and methods

Chlorosomes from the filamentous non-sulfur thermophilic green bacterium *C. aurantiacus* strain Ok-70-fl, cultivated anaerobically in batch cultures at 55°C [6], were prepared in 2 M NaSCN and 10 mM sodium ascorbate by standard methods [11] with minor modifications. Chlorosomes were isolated from three *C. aurantiacus* cell cultures with different BChl *c* contents. These cultures were grown at different light intensities: 5, 100, and 1000 W/m^2 (see Fig. 1).

Absorption and fluorescence excitation spectra of chlorosomes were measured with Hitachi-557 and Hitachi-850 spectrometers, respectively (Japan).

Femtosecond transient absorption measurements were carried out with a home-built amplified Ti:sapphire laser system with continuum generation, standard pump–probe scheme and optical multichannel analyzer (Oriel, France) detection [12] with modifications described below. Pump pulses were passed through an interference filter centered at 750 nm with a 10 nm spectral bandwidth. Probe pulses were of white light continuum. Cross-correlation function of the pump to that of the probe pulses showed ~110 fs pulse width. The energy

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Abbreviations: BChl, bacteriochlorophyll; ΔA, absorption difference

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ratio of pump–probe pulses was 100:1. The angle between the planes of polarization of pump and probe beams was 54.7° (magic angle). The operating frequency was 15 Hz. The resulting absorption difference (ΔA) spectra, obtained by averaging 5000–50 000 measurements at each delay, consisted of 500–1000 points. The optical density (OD) of samples was 0.5–1.0 (in a 1 mm thickness of a cuvette). The typical values of ΔA^{max}, shown in Figs. 2 and 3, were in the range of 10^{-5}–10^{-4} OD and were measured with an accuracy of 2–10%. An approximate estimation of excitation energy density gives a value $\sim 10^{12}$ photons/cm^2 per pulse corresponding to the pulse intensity, shown by the arrow in Fig. 4. All measurements were carried out at room temperature.

3. Results and discussion

All experiments were performed on chlorosomes isolated from three *C. aurantiacus* cell cultures grown at different light intensities which, as a consequence [6] had different ratios (η) of the absorption at the chlorosomal BChl *c* antenna maximum (740 nm) to that of the membrane core BChl *a* antenna (866 nm): $\eta = 10$, 16, and 30 (Fig. 1a). Thus, in the third culture ($\eta = 30$) the BChl *c* antenna size was approximately three times larger as compared to the first one ($\eta = 10$). The corresponding absorption spectra of chlorosomes, referred to as C-10, C-16, and C-30, are shown in Fig. 1b, c, and d, respectively.

The efficiencies of BChl *c* → BChl *a* excitation energy transfer in each sample estimated from the steady-state BChl *a* fluorescence excitation spectra, were shown to be 0.78 for C-10, and 0.80 for both C-16 and C-30 samples (Fig. 1b–d).

A unit BChl *c* aggregate of the chlorosomal antenna is thought to have a form of a cylindrical aggregate of excitonically coupled linear chains of BChl *c* oligomers [9,10]. The formation of oligomers results in a substantial increase in the oscillator strength of the Q_y absorption band (at the expense of the Soret band) compared to the monomeric pigments, and this effect (hyperchromism) was predicted for BChl/BPh *a* [13] and BChl *c* [14] to rise with increasing oligomer length. And this was the first we examined here for chlorosomes with different BChl *c* contents.

The hyperchromism (H) value for the BChl *c* Q_y band in each chlorosome sample was determined from the ratio of the oscillator strength of the Q_y transition band of oligomeric BChl *c* in vivo (740 nm band) to that of monomeric BChl *c* in vitro (660 nm band) (Fig. 1b–d). The monomeric BChl sample was obtained by acetone–methanol extraction of the chlorosome under study [11]. The H values were shown to rise with increasing BChl *c* content in chlorosomes: namely, $H = 1.55 \pm 0.03$, $H = 1.62 \pm 0.02$, and $H = 1.75 \pm 0.03$ for C-10, C-16, and C-30 chlorosomes, respectively, thereby showing a predicted progressive increase in the unit BChl *c* aggregate size as the growth light intensity was lowered [14].

This conclusion was examined in an independent experiment. Non-linear optical spectroscopy, being very sensitive to the aggregate size [15–23], is the most informative technique. We used a femtosecond transient absorption spectrometer described earlier [12].

Chlorosomes were excited using 110 fs pulses centered at 750 nm, at a repetition rate of 15 Hz, while the resulting broad-band ΔA spectra were monitored using white light probe pulses at different time delays. Figs. 2 and 3 show the room temperature isotropic ΔA spectra of chlorosomes measured in the spectral region from 710 to 830 nm at 200 fs delay, which corresponds to the maximal BChl *c* ΔA amplitudes for all samples near 750 nm (Fig. 2), and at 25, 35, and 100 ps delays, corresponding to the maximal BChl *a* ΔA amplitudes at 795 nm for C-10, C-16, and C-30 samples, respectively (Fig. 3).

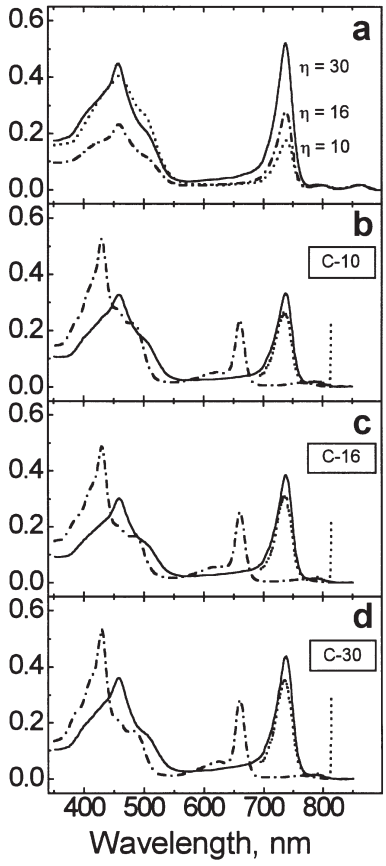

Fig. 1. Spectra of objects under study. a: Room temperature absorption spectra of three *C. aurantiacus* cell cultures, grown at 5 (solid line), 100 (dashed-dotted line), and 1000 (dashed line) W/m^2, in arbitrary units. The spectral quality of light (from incandescent lamps) was unchanged. The near-infrared maximum at 740 nm belongs to chlorosomal BChl *c*; the maxima at 808 and 866 nm belong to the membrane core BChl *a* antenna B808–866. All spectra are normalized to the absorption maximum at 866 nm since the size of the core B808–866 antenna is independent of growth conditions. The different BChl *c* contents in chlorosomes of these cultures are characterized by different ratios (η) of the absorption at 740 (A_{740}) and 866 (A_{866}) nm: $\eta = A_{740}/A_{866} = 10$ (dashed line), 16 (dashed-dotted line), and 30 (solid line). b, c, d: Absorption (solid lines) and BChl *a* fluorescence excitation (registration at 825 nm; dashed lines) spectra (normalized at 795 nm maxima) of chlorosomes, isolated from cells of Fig. 1a with $\eta = 10$ (b), $\eta = 16$ (c), and $\eta = 30$ (d), and referred to as C-10 (b), C-16 (c), and C-30 (d). The vertical line in each excitation spectrum corresponds to the signal at the wavelength of registration. The absorption maxima at 740 and 795 nm belong to chlorosomal BChl *c* and BChl *a*, respectively. Absorption spectra of monomeric BChl *c* in vitro (660 nm band), obtained by acetone–methanol (7/2, v/v) extraction of the chlorosome under study, are shown by dashed-dotted lines.

In these experiments, the effects of multiphoton processes were negligible since the excitation intensity used (shown by the arrow in Fig. 4) corresponded to the linear region of both BChl *c* and BChl *a* amplitude changes (Fig. 4). The shapes of the ΔA spectra showed no variations with excitation level within this linear region.

The resulting ΔA spectra presented in Fig. 2 were normal-

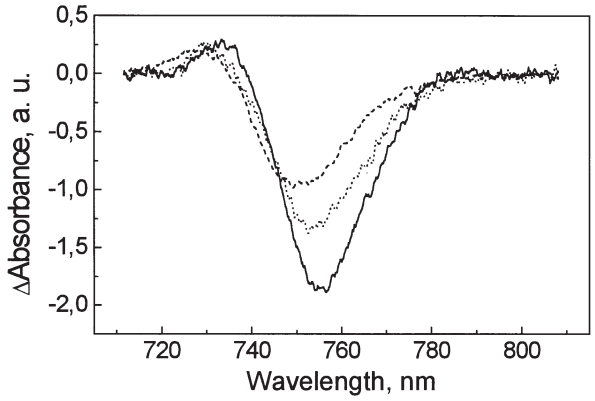

Fig. 2. Room temperature isotropic ΔA spectra of chlorosomes after 750 nm excitation at time delay 200 fs for C-10 (dashed lines), C-16 (dotted lines), and C-30 (solid lines) samples, respectively. All ΔA spectra are normalized to give the same final BChl *a* ΔA_{795} amplitude.

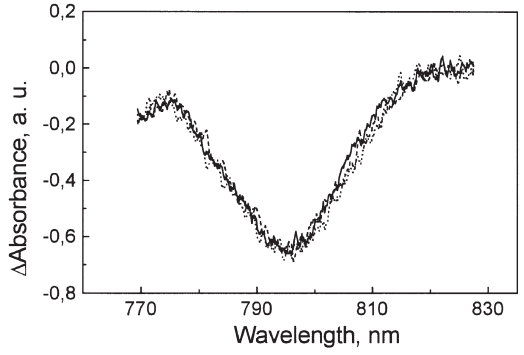

Fig. 3. Room temperature isotropic ΔA spectra of chlorosomes after 750 nm excitation at time delays 25, 35, and 100 ps for C-10 (dashed lines), C-16 (dotted lines), and C-30 (solid lines) samples, respectively. The delays were chosen to be equal to $\geq 2\tau$ according to experimental kinetics of BChl *c* → BChl *a* energy transfer (not shown) with $\tau = 11$ (for C-10), 17 (for C-16), and 40 ps (for C-30), depending on the antenna sizes [8]. All BChl *a* ΔA spectra are normalized to the ΔA maximum at 795 nm. The arbitrary units of ΔA scales in Fig. 2 are the same as in this figure. BChl *a* ΔA amplitudes were multiplied by a factor of 10.

ized to give the same final BChl *a* ΔA spectra in all samples under study. The principal features of each spectrum were similar to those measured earlier for chlorosomes from *C. aurantiacus* culture with a ratio $\eta = 8$ [11]. At 200 fs delay, each ΔA spectrum exhibited a BChl *c* photobleaching/stimulated emission (PB/SE) maximum near 750 nm, accompanied by a pronounced BChl *c* excited state absorption (ESA) maximum near 730 nm. When most of excitations were transferred from BChl *c* to BChl *a* (i.e. by 25, 35, and 100 ps for C-10, C-16, and C-30 samples, respectively), BChl *a* ΔA spectra developed, which exhibited only a PB/SE peak at 795 nm. The normalized spectra of three chlorosome samples clearly show the identity of BChl *a* ΔA spectra (Fig. 3). Thus, we concluded that the BChl *a* pigments in chlorosomes are functionally monomeric irrespective of the chlorosomal antenna size. The similar conclusion was made for the first time for the chlorosomal BChl *a* pigments from *C. aurantiacus* culture with a ratio $\eta = 8$ [11,24]. At the same time, a comparison of ΔA spectra measured near zero-time delay (Fig. 2) shows that an increased BChl *c* content is accompanied by an initial ΔA spectrum that is red shifted by up to 5 nm, narrower and up to 1.9 times greater in amplitude. The full width at half maximum (FWHM) of the ΔA spectrum decreases from 22 nm in the C-10 sample to 20 nm in C-30. Correction [11] for the efficiency of BChl *c* → BChl *a* excitation transfer and the hyperchromism of the aggregated BChl *c* Q_y band changes insignificantly the ratio of the initial BChl *c* bleaching amplitudes for the samples under study. The corrected ratio is $\Delta A_{C-10}:\Delta A_{C-16}:\Delta A_{C-30} = 1:1.35:1.75$, whereas the experimental ratio is $\Delta A_{C-10}:\Delta A_{C-16}:\Delta A_{C-30} = 1:1.38:1.93$ (Fig. 2).

All the fundamental changes in the initial BChl *c* ΔA spectra observed with increasing the BChl *c* content, i.e. a progressive increase in amplitude, red shift and narrowing of the bleaching band, are predicted by a theory of non-linear optical spectroscopy for (quasi-)linear molecular aggregates increasing in size and confirmed in a variety of experiments [15–23]. In particular, the principal changes in the initial BChl *c* ΔA spectra with increasing the aggregate size have recently been predicted for the chlorosomes under study assuming that the arrangement of pigments and their couplings remain unchanged in the process [23]. However, these spectral changes are observed only in the limit of a small aggregate. It is important that the shape of the difference absorption of the tubular aggregate of linear chains of BChl *c* oligomers with weak interchain interactions [8,23] is determined mostly by intrachain interactions [23]. As a result, the dependence of the shape and the amplitude of the difference absorption on the length (N) of a BChl *c* oligomer chain (N is the number of monomers per chain in a unit tubular aggregate) [23] is very similar to that for a quasi-linear aggregate [18–21]. In the tubular BChl *c* aggregates under study, N is not more than 5–6 [23,25], i.e. N corresponds to the limit of a small aggregate in a bacterial antenna ($N \leq 5$ [20]). In this limit, the bleaching amplitude increases proportionally to N [15,18,20,23]. The coherence (localization) size, which is actually probed by the non-linear absorption spectra, differs insignificantly from the physical size N of the aggregate. Thus, we concluded that the

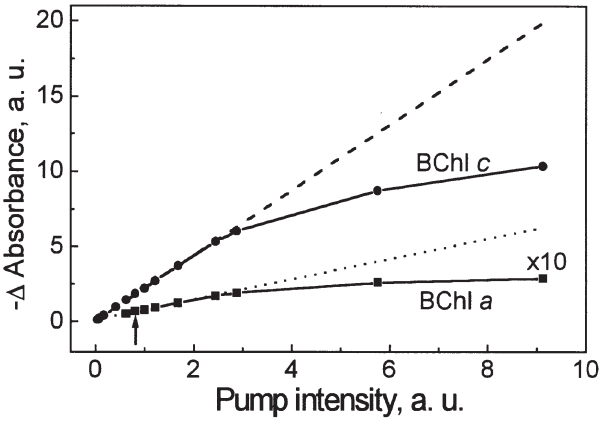

Fig. 4. The dependence on the pump intensity (excitation, at 750 nm) of the maximal ΔA amplitude of the signals representing BChl *c* (circles) and BChl *a* (squares) for the largest C-30 chlorosomes. The signals include both photobleaching and stimulated emission. BChl *a* ΔA amplitudes were multiplied by a factor of 10. All ΔA spectra of Figs. 2 and 3 were measured at a pump intensity, shown by the arrow, i.e. within the linear region of both BChl *c* and BChl *a* amplitudes changes where the experimental points lie on dashed or dotted straight lines. For the smaller size chlorosome the saturation intensity shifts to higher values.

observed difference in the amplitudes of the initial BChl *c* ΔA spectra demonstrates an increase in the unit BChl *c* aggregate size with increasing the antenna size.

Notice that in the opposite limit of a large aggregate ($N \gg 5$), the bleaching amplitude increases with N more slowly (if at all) and the spectral shift and narrowing of the bleaching band are practically absent [18,20]. In this limit, the coherence size can be much less than the physical size of the aggregate [16,20]. This case is typical for LH1/LH2 BChl *a* antenna aggregates of purple bacteria [16–21] and for B866 membrane BChl *a* antenna of the green bacterium *C. aurantiacus* [22].

In our experiments, the initial BChl *c* bleaching amplitude increased about twice as the chlorosomal antenna size grew approximately three times. This means (see above) that in chlorosomes under study, the unit BChl *c* aggregate size is about doubled as the chlorosomal antenna size is about trebled. A quantitative evaluation of the true sizes of the unit BChl *c* aggregates in the chlorosomal antennae requires additional experiments, which are in progress.

Our conclusion is in line with an earlier suggestion, based on *C. aurantiacus* chlorosome volume and BChl *c* content analysis, that the average BChl *c* packing density within the chlorosome is antenna size dependent [25]: in particular, it has been shown that the BChl *c* packing density grows almost twice as the BChl *c* content within the chlorosome increases about 3.5 times. In principle, this can be due to (i) variations in the packing density of rod elements within chlorosomes, or/and (ii) variations in the packing density of unit BChl *c* aggregates within a rod, or/and (iii) variations in the size of the unit BChl *c* aggregate within a rod. However, only the variations of the third type can provide both sets of findings made in this work. Quantitative comparison of our data with those of Golecki and Oelze [25] demonstrates unambiguously that the third mechanism makes a predominant contribution to the regulation of the BChl *c* packing density within the chlorosome, though involvement of the other mechanisms cannot be excluded.

Thus, we conclude that control over the size of a unit BChl *c* antenna aggregate helps this organism to survive over a wide range of light intensities. As the light intensity decreases, the size of the BChl *c* antenna increases [6] thus compensating light deficit by enhancing the light-absorbing capability of the antenna. An inevitable drop in the efficiency of the antenna functioning with increasing its size [1], in turn, is compensated by an increase in the size of the unit BChl *c* aggregate thus ensuring the high efficiency of energy transfer within the antenna regardless of its size.

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