Antenna size dependent exciton dynamics in the chlorosomal antenna of the green bacterium *Chloroflexus aurantiacus*

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Abstract Using picosecond fluorescence spectroscopy, we demonstrated antenna size dependent exciton dynamics in chlorosomal antenna, measured for intact cells of different cultures of the green bacterium *Chloroflexus aurantiacus* with different chlorosomal antenna size determined by electron microscopic examination of ultrathin sections of the cells. The measured bacteriochlorophyll (BChl) *c* excitation lifetimes show a quasilinear dependence on chlorosome size as predicted in our model for cylindrical exciton migration within the three-dimensional chlorosomal antenna. The migration model was developed for the proposed exciton model of chlorosomal BChl *c* aggregation. The data predict the time constant values for excitation energy transfer between BChl *c* aggregates as well as to BChl *a* of the baseplate.

Key words: Bacterial photosynthesis; Excitation energy transfer; Light-harvesting antenna; Green bacteria; Chlorosome

1. Introduction

The chlorosome contains several thousands of its main light harvesting pigments (BChl *c*, *d* or *e* depending on the species) associated with six subunits organized hexagonally in the form of hollow cylinders (called rod elements) each 5-10 nm in diameter and 100-250 nm long, with each chlorosome containing from 10 to 30 such rods running the length of the chlorosome [1]. The chlorosome antenna structure and functioning in different green bacteria have been extensively studied in the past decade [2]. However, a major question about the mechanism of excitation energy transfer within the chlorosome remains open. This problem is most acute in view of oligomeric BChl organization. The present work suggests a possible mechanism of excitation energy transfer within the chlorosome.

2. Materials and methods

All experiments were performed on intact cells of the thermophilic green bacterium *Chloroflexus aurantiacus* strain Ok-70-fl used in their own growth medium under strictly anaerobic conditions. The different size of chlorosomal antennae (i.e. the different ratios of BChl *c*/BChl *a* was adjusted by inhibiting the formation of BChl *c* by gabaculine [3].

Electron microscopic observations were made with a Hitachi-12 electron microscope (Japan), operating at 75 kV. For electron microscopy study, the cells were fixed in the culture medium with glutar-

aldehyde, postfixed with OsO_4, embedded in Epon-812, and ultrathin-sectioned by standard method [4]. Micrographs of the ultrathin sections were used for morphometric measurements (magnification $50\,000 \times 10$) to obtain histograms of chlorosomes heights and calculate the number of layers of rod elements in the chlorosome, assuming that the diameter of the rod equals 5.5 nm [4,5].

Absorption spectra of intact cells were recorded with a Hitachi-557 spectrophotometer (Japan).

Spectrally resolved fluorescence measurements for intact cells of *C. aurantiacus* were made with a picosecond spectrochronograph [6]. The pulse source was a mode-locked CW rhodamine 590 dye laser (pulse duration, 3 ps), synchronously pumped at 76 MHz by a Nd-Yag laser (Ontares, 'Coherent'). Emission, viewed at an angle of 90° to the exciting beam, was registered with a home-made synchroscan streak camera. The time resolution of the measuring system was 10 ps.

3. Results and discussion

Using the standard approach to the exciton-phonon problem in molecular crystals, we discovered that none of the earlier proposed models of BChl aggregation in chlorosomal antennae displays the in vivo exciton level structure of a BChl aggregate revealed by hole burning experiments on intact cells of different green bacteria [7–9].

All hitherto proposed molecular models of the chlorosome correspond to the two limiting cases of BChl chains packing: (i) noninteracting BChl chains (for review see [10,11]) and (ii) strongly exciton-coupled BChl chains with a high density of packing, postulating self-aggregation of pigments into a tubular macrocycle network [11,12]. For all these models, calculated absorption spectra consist of an intense line corresponding to the lowest exciton level and of weak lines corresponding to higher levels (not shown). In contrast, experimental hole burning spectra of chlorosomal antennae of green bacteria show a broad absorption spectrum due to many electronic (exciton) levels. The higher levels have ~98% of the total oscillator strength [7–9].

Alternatively, we proposed models of six linear exciton-coupled BChl chains with a low packing density, approximating that in vivo, to obtain the main spectral features found in natural antennae, i.e. the exciton level structure with intensive higher levels [7–9] and polarization of all levels parallel to the long axis of the chlorosome [6,13] (Fig. 1). It is suggested that the BChl-BChl interactions dominate only in determining the BChl chain structure. The spatial arrangement of the chains into tubular structures, with the interchain distance of about 2 nm predicted by our theory from experimental hole burning spectra, is assumed to involve another type of interactions.

The theory of excitation energy transfer within the chlorosome has been developed for our tubular aggregate model to describe antenna size dependent kinetics of fluorescence decay

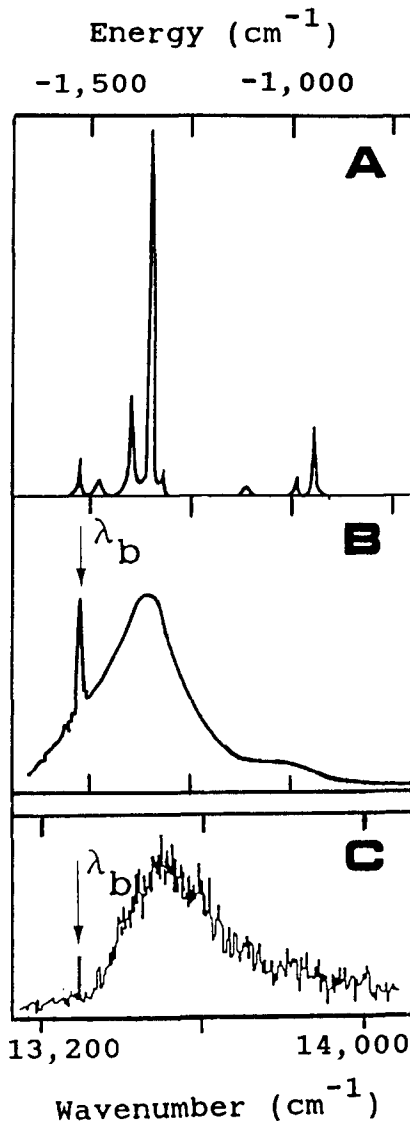*Corresponding author. Fax: (7) (95) 939 3181.

Energy (cm^{-1})

Fig. 1. (A) Typical calculated low-temperature absorption spectrum for six exciton-coupled chains model. Phonon frequency, $\Omega = 30$ cm^{-1}; homogeneous line width, $\Gamma/2 = 2$ cm^{-1}. (B) Calculated hole burning spectrum for the same conditions after introducing the 150 cm^{-1} homogeneous line width for higher levels and the 90 cm^{-1} inhomogeneous line width. For spectra A and B, the energy is counted from the monomer absorption maximum (upper scale). (C) Experimental hole burning spectrum for intact cells of *C. aurantiacus* at 1.8 K. Burning wavelength, λ_b, corresponds to the maximum of the lowest exciton level absorption band of BChl *c* (752 nm).

in chlorosomal antenna of *C. aurantiacus*. We used the three-dimensional model of the chlorosome proposed in [4]. The chlorosome contains *p* layers of rod elements packed in a hexagonal structure. Each rod element consists of tubular BChl *c* aggregates described above. Each aggregate from the first layer interacts with *q* BChl *a* molecules of the baseplate. The rate constant of energy transfer from an aggregate of the *p*-th layer to each of the two nearest aggregates of the $(p + 1)$-th layer is K_A^a; the rate of energy transfer from the BChl *c* tubular aggregate to the BChl *a* molecule of the baseplate is K_A^A; the rate of back transfer is K_a^A. The rates of the total losses in chlorosomal BChl *c* and BChl *a* antennae are K_c and K_a, respectively. The efficiency of the BChl *c* → BChl *a* energy transfer is determined by the efficiency of energy mi-

gration only along axis perpendicular to the membrane plane, hence, only by one chlorosome size, namely, by its height and, as a consequence, by the number of layers of rod elements, *p*.

According to our model, the energy transfer dynamics within the chlorosome implies the formation of a cylindrical exciton, delocalized over a tubular aggregate of six linear exciton-coupled BChl *c* chains, and Forster-type transfer of such a cylindrical exciton between nearest tubular BChl *c* aggregates as well as to BChl *a* of the baseplate. For this model, we obtained the analytical expression for the time constant of the fluorescence decay in the BChl *c* antenna as a function of the number of rod layers, *p*:

$$\tau(p) = (1 - \frac{(t^+/p)B_p}{(1 + t^+ + t^- + t(1 + t^-))D_{p-1} + t^2(1 + t^-)D_{p-2}})\tau_c$$

where $\tau_c = 1/K_c$; $D_p = -(1 + 2t)D_{p-1} - t^2 D_{p-2}$; $B_p = D_{p-1} - tB_{p-1}$; $D_0 = 1$; $D_1 = -(1 + t)$; $B_1 = 1$; $t = 2K_A^A/K_c$; $t^+ = qK_A^a/K_c$; $t^- = K_a^A/K_a$.

One may expect that all sizes of chlorosomes (not only those investigated in [14] but also the height of the chlorosome) are variable over some range in any cell culture. Then, the cells containing chlorosomes of different size *p*, can be characterized by the discrete distribution function $F(p)$, where *F* is the relative number of chlorosomes with *p* layers. In this case the time constant of the fluorescence decay in the BChl *c* antenna is:

$$\tau = \sum_p \tau(p)F(p)$$

If the $\tau(p)$ dependence is linear, then $\tau = \tau(<p>)$, where $<p> = \sum_p F(p)$.
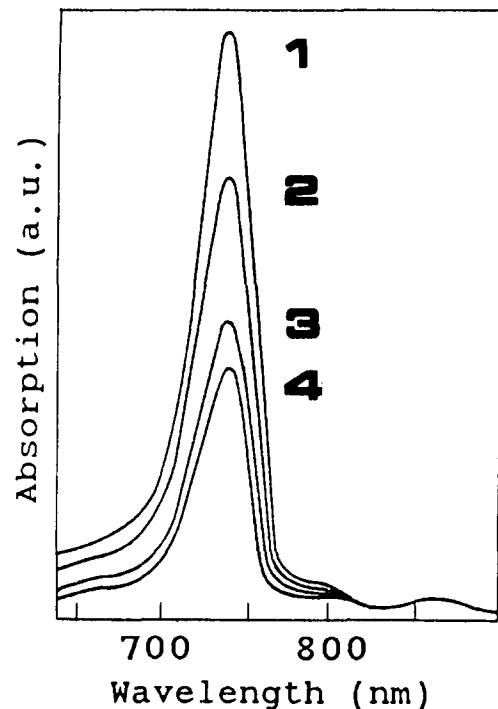

Fig. 2. Room-temperature near-infrared absorption spectra of intact cells of four *C. aurantiacus* cultures (Nos. 1–4) grown at the following concentrations of gabaculine: (1) 0 μM; (2) 0.7 μM; (3) 1.6 μM; (4) 2.2 μM. The maximum at 740 nm belongs to BChl *c* of the chlorosome; the maxima at ~800 and ~865 nm belong to BChl *a*.
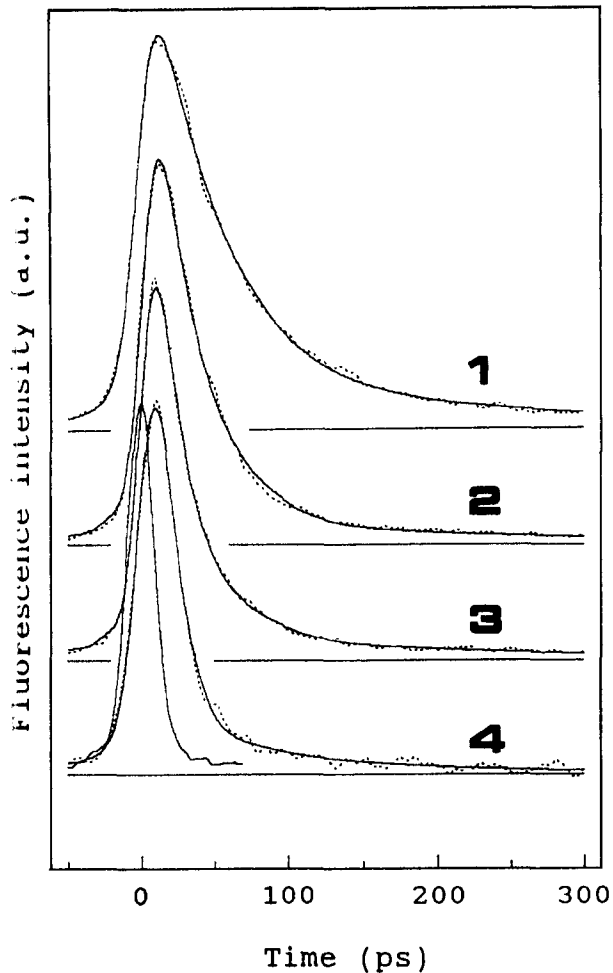
Fig. 3. Room-temperature isotropic picosecond fluorescence kinetics for BChl c fluorescence decay (dotted curves) in living cells for each of four C. aurantiacus culture shown in Fig. 2. The solid curves are the best multi-exponential fits to the data (an accuracy, 5%). Excitation was at 590 nm. The narrow profile (FWHM = 10 ps) is the apparatus response function.

We examined four cultures of C. aurantiacus cells with different BChl c content, as seen from their absorption spectra normalized at the wavelength of membrane BChl a absorption maximum, 865 nm (Fig. 2). Using electron microscopic examination of micrographs of ultrathin sections of these cells, we discovered that in each culture (and even in one and the same cell!) there are chlorosomes with different numbers of rod layers. It was shown that cells from each culture can be characterized by their own discrete distribution function $F(p)$. In line with the results obtained in [14], we have shown that cells with a higher BChl c content exhibit a larger amount of thicker chlorosomes.

The picosecond BChl c and BChl a fluorescence kinetics detected at the wavelengths of the corresponding fluorescence maxima, 750 and 880 nm, respectively, were measured for the same cultures. Fig. 3 shows the kinetics for BChl c emission which are strongly biphasic and characterized by a fast phase with lifetime τ_f = 37, 26, 17 and 12 ps for cultures No. 1, 2, 3 and 4, respectively, and by a slow phase with lifetime $\tau_s \sim 200$ ps for all cultures (the amplitude ratio $A_f/A_s \sim 15$). All kinetics for BChl a emission are monophasic with lifetime of ~ 200 ps for all cultures (not shown).

As should be expected, each culture is characterized by its own antenna size dependent time constant of BChl c fluorescence decay in chlorosomal antenna. Thus, the rate constant of fluorescence decay in the BChl c antenna (and, hence, the efficiency of excitation energy transfer from the BChl c antenna to the baseplate) decreases with increasing the chlorosome size, p, i.e. the number of rod layers. That is why the published values of BChl c fluorescence decay in whole cells exhibit lifetimes in a rather wide range, from 12 ps to ~ 40 ps ([6,15,16]; this work).

The membrane antenna size (the number of BChl a molecules per reaction center) remains unchanged [1] for all cultures under study, therefore, the BChl a fluorescence kinetics are also unchanged.

It should be stressed that in any real object one can register only the result of a simultaneous functioning of chlorosomal antennae which differ in their size p and, consequently, in times of BChl c fluorescence decay (for example, up to 4 times in cells of culture No. 1 containing chlorosomes with p = 1–4). Perhaps this fact forced researchers sometimes to suggest the existence of more than one pool of antenna pigments (2 pools at least in C. aurantiacus chlorosomes). Meanwhile, the hole burning spectra of intact cells completely exclude such an assumption.

In Fig. 4 the theoretical dependence $\tau(p)$ for BChl c fluorescence kinetics is shown for a set of parameters: $t = t^+ = 20$, $\tau_c = 275$ ps, $t^- = 0$. The $\tau(p)$ dependence is quasi-linear. This means that the $F(p)$ distribution can be characterized only by the mean value of p, $<p>$.

The experimental data for BChl c fluorescence kinetics and $<p>$ values determined for all cultures under investigation are summarized in Table 1 (only fast components reflecting the rate of BChl $c \rightarrow$ BChl a excitation energy transfer are presented). Fig. 4 demonstrates that theoretical and experimental exciton dynamics dependences on the chlorosome size, p, are in good agreement.

For the chosen set of parameters ($t = t^+ = 20$, $\tau_c = 275$ ps) we obtained $(K_A^A)^{-1} = 27.4$ ps; the hopping time between the antenna aggregates $(K_A^A)^{-1}/6 = 4.4$ ps (for the middle layers); $(K_A^A)^{-1}/4 = 6.6$ ps (for the upper layer); $(K_A^A)^{-1}/2 = 13.2$ ps (for one layer of rods). The time of energy transfer to the BChl a of the baseplate $(qK_A^a)^{-1} = 13.7$ ps, with this value
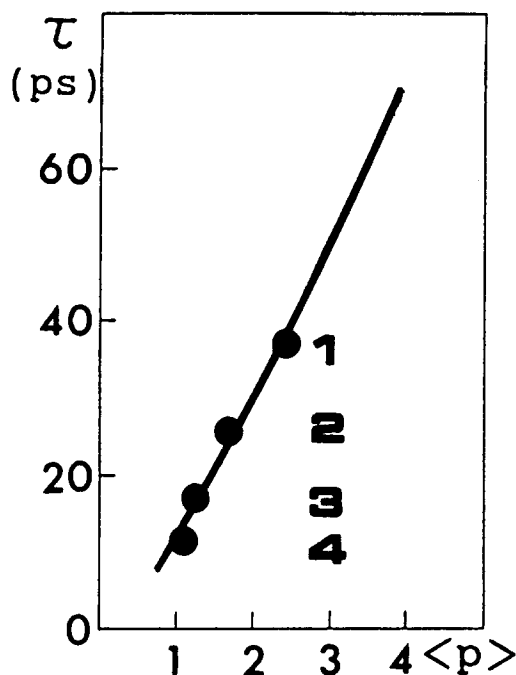
Table 1
Experimental data for BChl c fluorescence kinetics, τ_f, and the $F(p)$ and $<p>$ values determined for C. aurantiacus intact cells from four cultures shown in Fig. 2

Culture No.	τ (ps)	$F(p)$				$<p>$
		1	2	3	4	
No. 1	37	0.01	0.35	0.54	0.02	2.4
No. 2	26	0.42	0.63	0.01	0.00	1.7
No. 3	17	0.75	0.23	0.00	0.00	1.2
No. 4	12	0.90	0.10	0.00	0.00	1.1

Fig. 4. Theoretical dependence $\tau(p)$ (solid curve) and experimental points $\tau(<p>)$ for BChl c fluorescence decay in living cells of each of four *C. aurantiacus* culture shown in Figs. 2 and 3.

being very close to the measured $\tau = 12$ ps value for the culture No. 4 predominantly with one layer of rods.

Thus, the data suggest a possible mechanism of excitation energy transfer within the chlorosome, based on the exciton model of BChl c aggregation proposed by us.

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