Exciton levels structure of antenna bacteriochlorophyll *c* aggregates in the green bacterium *Chloroflexus aurantiacus* as probed by 1.8–293 K fluorescence spectroscopy

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Abstract We have demonstrated temperature-dependence of the steady-state fluorescence lineshape of the bacteriochlorophyll (BChl) *c* band measured for intact cells of the green bacterium *Chloroflexus aurantiacus* over the 1.8–293 K range. The measured temperature-dependence has been shown to be in good agreement with the theoretical one, calculated for our original model of pigment organization in the chlorosomal oligomeric antenna of green photosynthetic bacteria based on spectral hole-burning studies (Fetisova, Z.G. et al. (1996) Biophys. J. 71, 995–1010). This model implies that the BChl *c* antenna unit is a tubular aggregate of six exciton-coupled linear pigment chains having the exciton level structure with strongly allowed higher levels.

Key words: Bacterial photosynthesis; Antenna pigment organization; Bacteriochlorophyll *c*; Green bacterium

1. Introduction

The chlorosomal extramembrane antenna of green bacteria contains several thousand main light harvesting pigments in aggregated state (bacteriochlorophyll (BChl) *c*/*d*/*e*, depending on species) associated with six subunits organized hexagonally in the form of hollow cylinders (called rod elements), each 5–10 nm in diameter and about 100–250 nm long. The chlorosome contains 10–30 such rods running in the length of each chlorosome. Besides BChl *c*/*d*/*e*, the chlorosome contains a small amount of BChl *a* which is thought to be located in the base plate, connecting the chlorosome to the cytoplasmic membrane in which the B808-866 BChl *a* antenna and reaction centers are located (for review see [1]).

The strong orientational ordering of the BChl *c* Q_y transition dipoles along the long axis of the chlorosome, demonstrated both in situ [2] and in isolated antenna complexes [3–5], suggests that an elementary BChl aggregate has the form of at least a quasi-linear chain. A lot of models of chlorosomal BChl aggregates have been proposed (for review see [1]). Hole-burning spectroscopy studies [6–8] allowed us for the first time to obtain fundamental information on the fine structure of electronic spectra of oligomeric BChl *c*/*e* in vivo that provided us with an exciton level structure criterion for theoretical evaluation of any model for pigment aggregation in the chlorosome [9]. It was shown that none of the hitherto proposed molecular models of BChl aggregation in the chlorosome, corresponding to the two limiting cases of BChl chains packing: (1) non-interacting BChl chains and (2) strongly exciton-coupled BChl chains with a high density of packing, postulating self-aggregation of pigments into a tubular macrocycle network (containing ~20–40 pigment chains) [10,11], displays the in vivo exciton level structure of a BChl aggregate revealed by hole-burning spectroscopy [9]. For all these models, calculated absorption spectra consist of an intensive line, corresponding to the lowest exciton level, and of weak lines corresponding to higher levels. 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 [6–8]. Alternatively, a new original model of aggregation of chlorosomal pigments was proposed [9]. This model of six exciton-coupled linear BChl *c*/*d*/*e* chains, arranged in a tubular shape, with a low packing density, approximating that in vivo, was generated to yield the key spectral features found in natural antennae, i.e. the exciton level structure with strongly allowed higher levels, revealed by spectral hole-burning experiments [6–8], and polarization of all the levels parallel to the long axis of the chlorosome [2–5].

Thus, the exciton level structure of the chlorosomal BChl *c* aggregate in our model fundamentally differs from that in all other models [9]. Based on the exciton level structure criterion, the adequacy of a model of BChl *c* aggregation can also be tested by the temperature-dependence of the steady-state BChl *c* fluorescence lineshape.

It is obvious that for all earlier models of a BChl *c* aggregate, the steady-state fluorescence lineshape of the BChl *c* band is temperature-independent, since the BChl *c* fluorescence band at any temperature comprises a single intensive line, belonging to the lowest exciton level of the aggregate.

In contrast, the steady-state fluorescence lineshape of the BChl *c* aggregate in vivo as well as that of the model tubular BChl *c* aggregate of six exciton-coupled linear chains with intensive higher levels [9] are expected to be strongly temperature-dependent, since the population of different exciton levels is dramatically changed with the temperature.

In this work, we demonstrate the temperature-dependence of the steady-state fluorescence lineshape of the BChl *c* band, measured for *Chloroflexus aurantiacus* intact cells over the 1.8–293 K range manifesting the adequacy of our original model [9] of BChl *c* organization in the chlorosome.

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Abbreviations: BChl, bacteriochlorophyll; ZPH, zero phonon hole

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2. Materials and methods

All experiments were performed on intact 2 days old cells of the thermophilic green bacterium *C. aurantiacus* (strain Ok-70-fl), used in their own growth medium under strictly anaerobic conditions. Fluorescence excitation spectra as well as hole-burning experiments were carried out with a home-built CW dye laser (linewidth 0.5 cm), tunable with a three-plate birefringent filter as described earlier [6]. The spectral hole-burning measurements were carried out in superfluid helium at 1.8 K. Fluorescence was recorded through a MDR-2 monochromator (dispersion 4 nm/mm) using a red-sensitive photomultiplier RCA C31034 A-02 in the photon counting mode. The temperature was measured by a Cu-Fe thermocouple with an accuracy of 0.1 K and was controlled by regulating the helium gas flow through the sample chamber.

3. Results and discussion

Fig. 1 shows the 1.8 K fluorescence excitation spectrum for *C. aurantiacus* intact cells measured at the wavelength of the BChl *a* fluorescence band maximum, 820 nm. The band with a maximum at 742.8 nm belongs to BChl *c*. The 0-0 transition band of the lowest exciton state of the BChl *c* aggregate in vivo was detected by zero phonon hole (ZPH) action spectroscopy, as described earlier [6], and separated at 1.6 K as the lowest energy 752.4 nm band of the near-infrared excitation spectrum. These data confirmed our previous ones [6], demonstrating that (1) the BChl *c* absorption lineshape is determined by higher exciton levels, which are homogeneously broadened due to relaxation and have the greater part of the total dipole strength of the BChl *c* Q_y transition, (2) the lowest exciton level, 170 cm^{-1} red-shifted with respect to the absorption maximum, is inhomogeneously broadened (FWHM = 90 cm^{-1}) and has no more than 2% of the total dipole strength of the BChl *c* Q_y transition.

Steady-state fluorescence of the BChl *c* band (maxima at 750–760 nm) measured for the same intact cells of *C. aurantiacus* upon excitation in the BChl *c* Q_y transition band (at 720 nm) over the 1.8–293 K temperature range is shown in Fig. 2. The minor bands are due to fluorescence of BChl *a*. The same fluorescence spectra, normalized to the BChl *c* maxima, are shown in Fig. 3A. As should be expected, these data

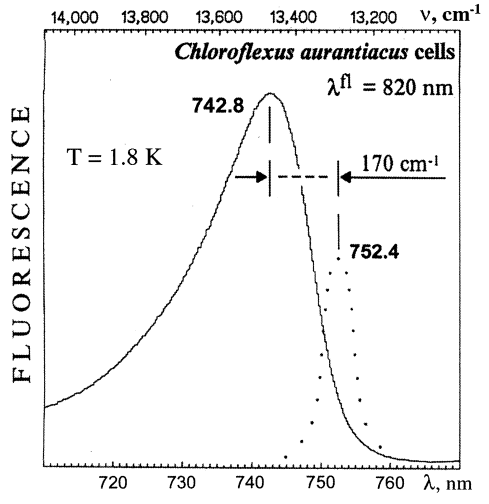

Fig. 1. 1.8 K fluorescence excitation spectrum for *C. aurantiacus* cells. The dotted curve is the 0-0 transition band of the lowest exciton state of the BChl *c* aggregate detected by ZPH action spectroscopy (shown on an expanded scale).

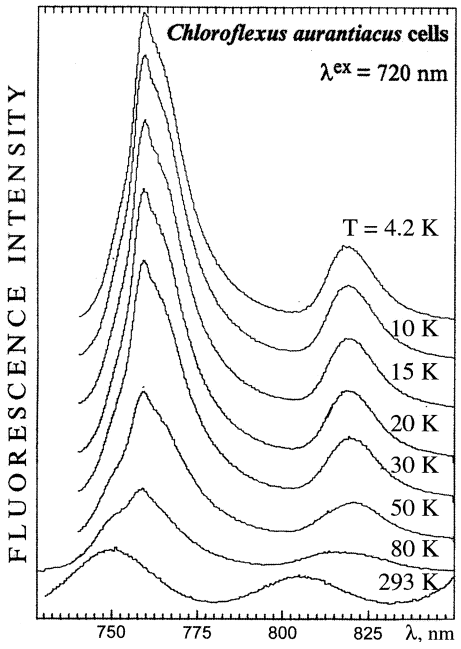

Fig. 2. Temperature-dependence of the steady-state BChl *c* fluorescence, measured for *C. aurantiacus* intact cells over the 1.8–293 K range.

clearly show that the steady-state fluorescence lineshape of the BChl *c* band in vivo is strongly temperature-dependent.

It is interesting that if a change in temperature leaves the spectral position of exciton levels of BChl *c* aggregates unaltered (as could be expected looking at the spectra of Fig. 3A), then, the energy gap between the fluorescence maxima at room and liquid helium temperatures would be expected to equal the energy gap between the lowest exciton level and the most intensive one in the absorption spectrum. Our hole-burning and fluorescence experiments, performed on one and the same cell culture, have shown that both of the energy gaps of interest are equal to 170 cm^{-1} (compare Figs. 1 and 3A).

A theoretical simulation of the BChl *c* fluorescence lineshape has been performed for our tubular exciton model [9]. Consider that the cylinder is formed by L parallel linear pigment chains. Each chain contains N BChl *c* molecules. n is the number of a molecule in a row, l is the number of the row, $\overline{d}_{nl}$ is the transition dipole moment of the nth molecule from the lth row. Exciton states are characterized by two numbers, k and κ, where k takes N integer values 1,2,...N, κ takes L integer values $0, \pm 1, \pm 2,...$. There are L Davydov components having the exciton structure similar to that of the linear chain. The energies and the dipole moments of the (k, κ) exciton state are equal to:

$$E_{k\kappa} = \Delta E + 2M\cos k\theta + 2M'\cos \kappa\Phi \quad (1)$$

$$\vec{d}_{k\kappa} = \sum_{n=1}^{N}\sum_{l=1}^{L} c_n^k c_l^\kappa \vec{d}_{nl};$$

$$c_n^k = (2/(N+1))^{1/2}\sin(\theta nk);\ \theta = \pi/(N+1);\ ;$$

$$c_l^\kappa = (1/L)^{1/2}\exp(i\Phi l\kappa);\ \Phi = 2\pi/L \quad (2)$$

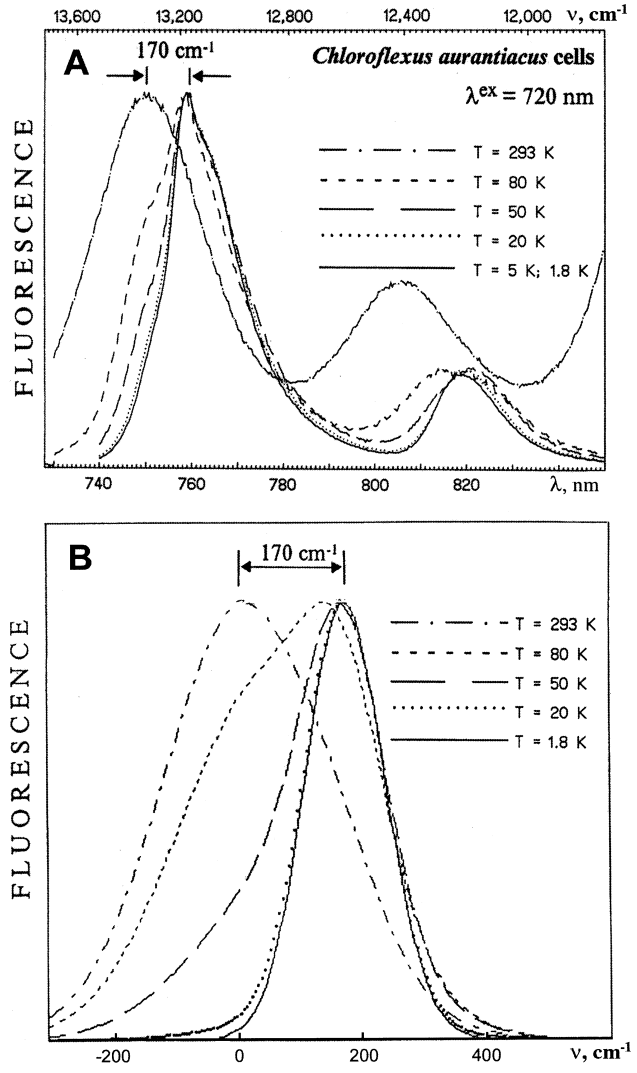

Fig. 3. Temperature-dependence of the steady-state BChl *c* fluorescence lineshape measured for *C. aurantiacus* cells (A) and calculated for our original model of BChl *c* organization in the chlorosome (B) over the 1.8–293 K range (see the text).

where ΔE is the energy of electronic excitation of the BChl monomer, M is the interaction energy between molecules n and $n+1$, belonging to the same row. The M' value, characterizing the splitting between Davydov components, equals:

$$M' = \sum_j M'_j (N+1)^{-1} (\sin(\theta kj)\mathrm{ctg}(\theta k) + (N+1-j)\cos(\theta kj));$$
$$M'_j = M'_{n-m};\ j = 0,\ \pm 1,\ \ldots\ \pm(N-1) \qquad (3)$$

where n and m correspond to the molecules from the nearest neighboring rows, M' describes the interaction between molecules from different rows. Note that $M'_{nm} = M'_{n-m}$ due to the translational symmetry. In our tubular model, $L = 6$, $N \leq 6$ [9], in accordance to the in vivo BChl *c* packing density [12]. To explain the spectral features revealed by spectral hole-burning experiments in the framework of our tubular model, we must assume that M is ~ -750 cm^{-1} and M' is 40–45 cm^{-1}. In this case, the energy gap between the dipole allowed for a $\kappa = 0$ level and the lowest $\kappa = L/2$ level (weakly non-forbidden due to the site inhomogeneity) is $4M' \approx 170$ cm^{-1} [9]. The fluorescence spectrum of the BChl *c* tubular aggregate can be determined as:

$$F(E) = \sum_{k,\kappa} d^2_{k\kappa} p_{k\kappa} \exp\left(-(E - E_{k\kappa} + S_{k\kappa})^2 / W^2_{k\kappa}\right);$$
$$p_{k\kappa} = \text{const} \cdot \exp(-E_{k\kappa}/k_B T) \qquad (4)$$

where $p_{k\kappa}$ is the steady-state population for the (k,κ) level, $W_{k\kappa}$ is the linewidth (FWHM is $W'_{k\kappa} = W_{k\kappa}2\sqrt{\ln 2}$) determined by the homogeneous width (due to phonon wings and the relaxation to the lowest exciton level) as well as by the inhomogeneous width. $S_{k\kappa}$ is the Stokes shift.

Temperature-dependence of the steady-state BChl *c* fluorescence lineshape, calculated at the same temperatures (see Fig. 3A) for our original model of pigment organization in the chlorosomal antenna [9], is shown in Fig. 3B. Comparison of experimental and calculated temperature-dependent BChl *c* fluorescence spectra (Fig. 3A and B) shows that all of the fundamental features of experimental spectra are described to a good approximation by the exciton theory of spectra developed for our original model of BChl *c* aggregation in the chlorosome. The best fit of experimental spectra to calculated ones has been obtained for $S_{k\kappa} = 110$ cm^{-1} for all the levels, $W'_{k\kappa} = 150$ cm^{-1} for the lowest level, $W'_{k\kappa} = 300$ cm^{-1} for all higher levels, when the relative intensities of Davydov components $\kappa = 0$, $\kappa = \pm 1$, $\kappa = \pm 2$ and $\kappa = 3$ are 100, 27, 6 and 4, respectively. This set of parameters is in good agreement with that used in our hole-burning spectra simulation [9].

At room temperature, only the $k = 1$ level of the $\kappa = 0$, $\kappa = \pm 1$, $\kappa = \pm 2$ and $\kappa = 3$ components (corresponding to the 742.8, 745.3, 749.9 and 752.4 nm absorption lines, respectively [9]) is populated and the BChl *c* fluorescence spectrum is essentially determined by the strongest $\kappa = 0$ component, corresponding to the band maximum. At 1.8 K, only the $\kappa = 3$ component (corresponding to the lowest exciton level) is populated and the corresponding line in the BChl *c* fluorescence spectrum is remarkably red-shifted with respect to that at room temperature. At intermediate temperatures, the BChl *c* fluorescence spectrum is multicomponent. Note that the $\kappa = 0$ component is close to the $\kappa = \pm 1$ one as well as the $\kappa = \pm 2$ component is close to the $\kappa = 3$ one [9]. For this reason, the spectrum looks like a two-component one.

Thus, the temperature-dependence of the steady-state BChl *c* fluorescence lineshape for *C. aurantiacus* cells measured over the 1.8–293 K range is in good agreement with our original model of BChl *c* organization in the chlorosome [9].

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