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Experimental evidence of oligomeric organization of antenna bacteriochlorophyll *c* in green bacterium *Chloroflexus aurantiacus* by spectral hole burning

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Spectral hole burning has been used to prove experimentally the existence in natural antenna of one of the predicted structural optimizing factors — antenna pigment oligomerization [J. Theor. Biol. 140 (1989) 167] — ensuring high efficiency of excitation energy transfer from antenna to reaction center. This point has been examined for the chlorosomal antenna of green bacterium *Chloroflexus aurantiacus* by hole burning in fluorescence excitation and emission spectra of intact cells at 1.8 K. The persistent hole spectra have been found to be consistent with a strongly exciton-coupled bacteriochlorophyll *c* (BChl *c*) chromophore system. The lowest exciton state of BChl *c* oligomers has been directly detected and separated as the lowest energy inhomogeneously broadened band (FWHM ~90 cm^{-1}, position of maximum, at ~752 nm) from the near-infrared BChl *c* band (FWHM ~350 cm^{-1}, position of maximum, at ~742 nm) of 1.8 K excitation spectrum.

Light-harvesting antenna; Pigment oligomerization; Energy transfer; *Chloroflexus aurantiacus* cell

1. INTRODUCTION

The photosynthetic apparatus structure must be highly optimized in vivo to operate with the observed 90% quantum yield of primary photochemistry [1]. The strategy of our experimental studies [2–6] comprises purposeful searching in natural antennae for structural optimizing factors which we have found theoretically [1,3,7–10]. In this work we have made an attempt to prove experimentally the existence in natural antennae of one of the predicted structural optimizing factors [9,10], antenna pigment oligomerization.

We have studied the green bacterium *Chloroflexus aurantiacus*. The largest part of antenna pigments of the bacterium is localized in the so-called chlorosomes: ellipsoid bodies attached to the inside of the cytoplasmic membrane [11]. Chlorosomes contain the major antenna pigment, bacteriochlorophyll *c* (BChl *c*), whereas RCs and the greater part of the antenna BChl *a* are associated with the cytoplasmic membrane [11]. It is in the larger chlorosomal BChl *c* antenna that one could expect realization of this design principle, antenna pigment oligomerization [1]. To solve the problem under investigation the spectral hole burning method has been chosen as the most informative since at helium temperatures there is no uphill energy transfer and, any destructive process destroys all the molecules of an oligomer together as a unit.

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2. EXPERIMENTAL

Chloroflexus aurantiacus strain B-III was grown anaerobically at 57°C. All the experiments were performed on intact 2-day-old cells in their growth medium at 1.8 K. The 1-mm thick samples had an optical absorbance at BChl *c* absorption maximum of OD ~ 0.3. The irradiated sample area was several square millimeters.

The fluorescence excitation spectra were recorded and burning was performed with a home-built CW dye laser (line width 0.5 cm^{-1}). The dye Oxazine-1 was used and a Coherent CR-2000 K Kr^+-laser served as a pump. The fluorescence measured in the reflection mode was recorded with a DFS-24 double spectrometer (dispersion 0.45 nm/mm, typical slit widths 0.1–0.2 mm) using a photomultiplier RCA C31034 A-02 in a photon counting regime. Burning powers varied from 0.5 mW/cm^2 to 5 W/cm^2, and burning times were from 250 s to 65 min. The spectral holes were probed by scanning the laser attenuated by a factor of 1000 over the spectral region investigated.

3. RESULTS

Figs. 1 and 2 summarize the results of a whole series of experiments on the persistent hole burning in the BChl *c* band (maximum at ~742 nm) of the BChl *a* fluorescence excitation spectrum. Varying the wavelength of laser irradiation we have found that:

(1) At any burning wavelength within a wide short-wave spectral range (from the short-wave edge up to the wavelength corresponding to the ~0.7-height amplitude on the long-wave slope of the excitation

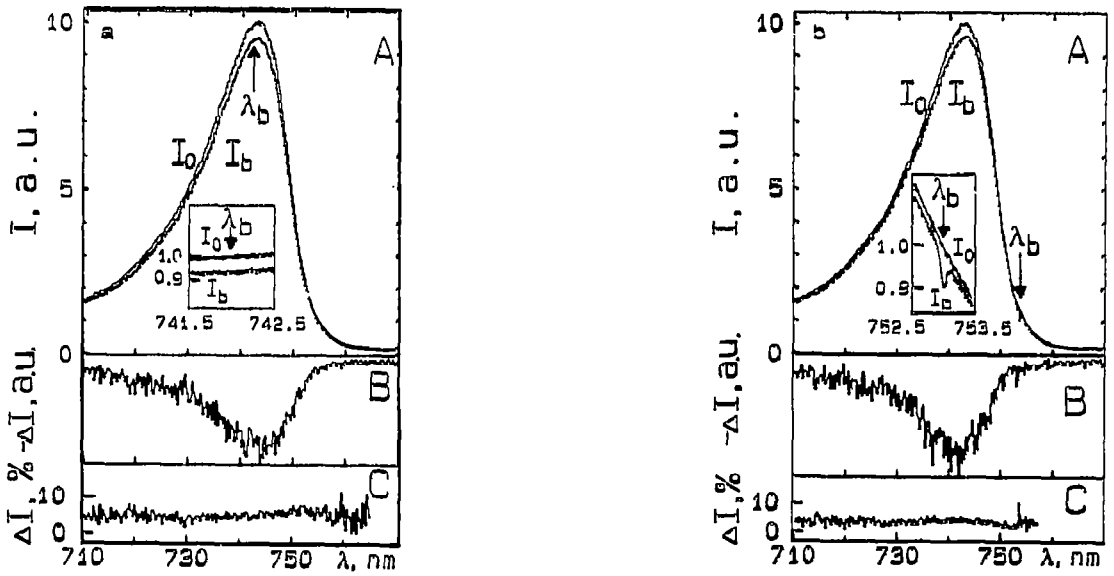

Fig. 1. 1.8 K hole-burned excitation spectra of *C. aurantiacus* cells at (a) λ_b = 742 nm; (b) λ_b = 753 nm. The λ_b is shown by the arrow and the insert represents expanded scan around this position. (A) Excitation spectrum before (I_0) and after (I_b) burning; (B) difference hole-burned spectrum (in arb. units): ΔI(a.u.) = I_0–I_b; (C) difference hole-burned spectrum (in %): ΔI(%) = (I_0–I_b)/I_0. Registration, at 820 nm. Burn conditions were: (a) λ_b = 742 nm: 16 min at 1 W/cm²; (b) λ_b = 753 nm; 16 min at 20 mW/cm².

band), the difference hole-burned excitation spectrum displays a broad non-resonant hole reproducing the contour of the whole excitation spectrum (Fig. 1a);

(2) At any burning wavelength within a range of the long-wave slope of the excitation band (from the wavelength corresponding to the ~0.7-height amplitude on the long-wave slope of the excitation band up to the long-wave edge of the band), the difference hole-burned excitation spectrum displays both (Fig. 1b), (a) a sharp resonant zero-phonon hole (ZPH), and (b) a broad non-resonant hole produced with the same λ_b; the contour of the spectrum of this non-resonant hole always reproduces the contour of the whole excitation spectrum.

(3) We did not observe holes deeper than 30%.

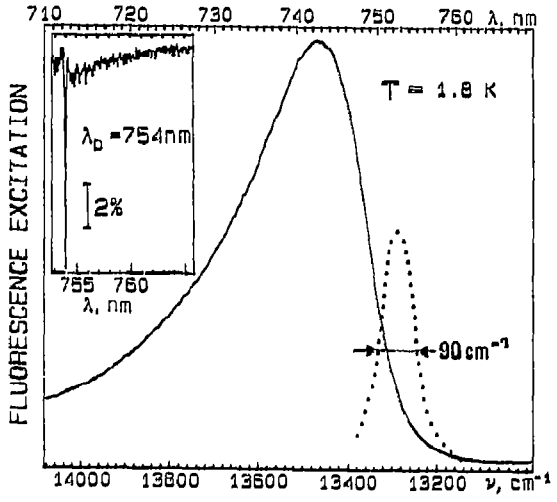

Fig. 2. 1.8 K near-infrared fluorescence excitation uncorrected spectra of intact cells of *C. aurantiacus*. Registration, at 820 nm. The dotted curve is the 1.8 K spectrum of the burnt ~752 nm band of the excitation spectrum on an expanded scale (see text). (Insert) Difference hole-burned excitation spectrum of intact cells of *C. aurantiacus* at 5 K. Burn conditions are: λ_b = 754 nm, 23 min at 150 mW/cm².

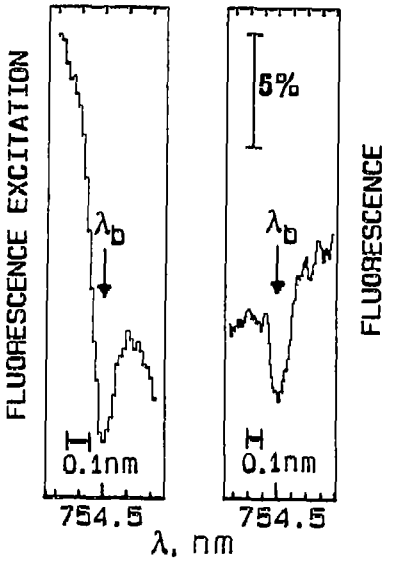

Fig. 3. Difference hole-burned spectra displaying resonant ZPH burnt simultaneously with the same λ_b in fluorescence and excitation spectra of intact cells of *C. aurantiacus* at 5 K. Excitation, at 720 nm. Registration, at 820 nm. Burn conditions are: λ_b = 754.5 nm; 8 min at 70 mW/cm².

(4) Measuring the depth of the burnt ZPH as a function of burning wavelength at a constant burning dose one can find the spectrum of the burnt band (plotted as black dots on an expanded scale in Fig. 2). The lowest-energy BChl *c* band has the following characteristics: (i) the bandwidth is ~90 cm^{-1}; (ii) the maximum is located at ~752 nm; (iii) the widths of ZPH (FWHM = 1 cm^{-1}, uncorrected for 0.5 cm^{-1} read resolution) were found to be independent of the burning wavelength (though, perhaps, they are laser-limited).

(5) Resonant ZPH are burnt simultaneously in both excitation and fluorescence spectra (Fig. 3). This means that the observed ZPH correspond to zero-phonon lines of 0–0 transitions, and the lowest energy BChl *c* band is essentially of purely electronic nature.

Since ZPH track λ_b, the 90 cm^{-1} FWHM of the lowest energy BChl *c* band is largely determined by inhomogeneous broadening.

4. DISCUSSION

The above findings were shown to be consistent with the BChl *c* organization in chlorosomes as BChl *c* oligomers. Indeed, if the chlorosomal antenna consists of oligomers, one can assume that its absorption or fluorescence excitation spectrum consists of strongly overlapping inhomogeneously broadened bands due to separate exciton components. If the near-infrared BChl *c* band were a superposition not only of the bands of 0–0 transitions of different exciton components of an oligomer but also of their vibrational bands, the results of our steady-state experiments would not alter. The presence itself of several bands in the spectrum (as well as their excitonic nature) is confirmed by the facts, that in the near-infrared BChl *c* absorption region, the degree of linear dichroism is not constant [12], the circular dichroism spectrum exhibits a large derivative-shaped feature [13], the fourth derivative spectrum of living cells clearly detects more than 8 bands, their widths larger than their energy splittings [14], and, at last, we have succeeded in direct separation of the 0–0 band of the lowest exciton component, 752 nm band (this work). The above assumption is valid within the limits of weak exciton–phonon coupling of the optical transitions associated with this antenna absorption system and inhomogeneous broadening of the absorption component profiles (both features are found to be common for all photosynthetic antenna systems studied [15]). For the 752 nm band, weak coupling is manifested by the small intensity of the pseudo-phonon sideband (Fig. 2, insert) and, the magnitude of inhomogeneous broadening was shown to be equal to 90 cm^{-1}. As to the higher energy exciton components, our main conclusion is independent of their bands being homogeneously or inhomogeneously broadened.

If there is no correlation between the energies of the exciton states of different oligomers, then at any burning wavelength, λ_b, (i) any level with an energy smaller than λ_b can be burned since all the levels are populated due to efficient downhill energy transfer between inhomogeneously broadened exciton states of different oligomers, and (ii) any level of such a system with an energy higher than λ_b can be burned (and will inevitably be burned) as an upper exciton level of a burnt oligomer excited either directly or via energy transfer. Thus, in such a system with efficient excitation migration within the whole chlorosome, there is always a probability for any oligomer of the chlorosome to be excited at any excitation wavelength. Upon burning the spectrum of an oligomer disappears from the common spectrum of the ensemble as a unit. This leads to the burning of the whole contour of the near-infrared spectrum of such a system in our steady-state experiment at any excitation wavelength.

When exciting higher exciton components, both fast downward scattering to any lowest energy levels within the lowest inhomogeneous exciton band (again due to lack of correlation between their energies), and the efficient energy transfer between all the exciton states of different oligomers, do not allow for selectively burning either a single higher exciton level or the lowest one. As a consequence, we observed only a broad hole reproducing the contour of the whole near-infrared excitation spectrum.

It is obvious that in such a system zero-phonon holes can be burned only in the spectral range of the lowest exciton band upon burning into it. Indeed, when directly exciting the lowest exciton component (together with the overlapping higher one(s)), we observed simultaneous burning of both, a resonant narrow spectral hole due to the zero-phonon character of the transitions corresponding to the lowest energy BChl *c* band in the excitation spectrum and a broad non-resonant spectral hole reproducing the contour of the whole near-infrared excitation spectrum due to an excitonically coupled BChl *c* chromophore system as described above.

In the opposite case of weak interactions between BChl *c* antenna molecules, i.e. if all the bands of the spectrum investigated were due to uncoupled BChl *c* monomers, only a sharp resonant hole due to the zero-phonon line would be observed in the lowest energy band upon burning into it, since there is no uphill energy transfer at helium temperatures.

Hence the persistent hole spectra are found to be consistent with a strongly coupled BChl *c* chromophore system within a cluster.

Thus, the oligomerization of the pigment molecules in natural light-harvesting antennae should be considered as one of the optimizing structural factors [1,9,10] ensuring high efficiency of excitation energy transfer from antenna to reaction center.

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