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Spectral hole burning study of intact cells of green bacterium *Chlorobium limicola*

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Spectral hole burning studies of intact cells of the green bacterium, *Chlorobium limicola*, have proven that the Q_y-absorption system of antenna bacteriochlorophyll *c* (BChl *c*) should be interpreted in terms of the delocalized exciton level structure of an oligomer. For the first time the 0–0 band of the lowest exciton state of BChl *c* oligomers has been directly detected as the lowest energy inhomogeneously broadened band (FWHM ~100 cm^{-1}; position of maximum, at ~774 nm) of the near-infrared BChl *c* band of 1.8K excitation spectrum (FWHM = 830 cm^{-1}; position of maximum, at 751 nm).

Light-harvesting antenna; Pigment oligomerization; Energy transfer; *Chlorobium limicola* cell

1. INTRODUCTION

We have studied the organization of the pigments in the light-harvesting antenna of the green sulfur bacterium *Chlorobium limicola*. This light-harvesting antenna is an order of magnitude larger than in purple bacteria, and several-fold larger than in higher plants. The largest part of antenna pigments is localized in extra-membrane antenna structures called chlorosomes containing the major antenna pigment – bacteriochlorophyll *c* (BChl *c*) (~1500 molecules per one reaction center). Reaction centers and the grCeater part of the antenna BChl *a* are associated with the cytoplasmic membrane [1]. Excitation energy transfer from BChl *c* to BChl *a* is very rapid and highly efficient [2–4]. The possible importance of delocalized exciton states for the efficiency of energy transfer process has been considered by us in [5,6]. Moreover, possible BChl *c* organization in vivo has been suggested to be oligomers proceeding from the results of a comparative analysis of spectral features of chlorosomes and BChl *c* oligomers in vitro [7]. However, no direct proof for natural chlorosome of *C. limicola* has been found yet. Recently it has been shown that one of the most suitable method for such investigation is spectral hole burning [8]. The method of hole burning in the BChl *c* band of the BChl *a* fluorescence excitation spectrum of intact cells has been chosen. This is the most informative technique because, first, it could be applied to intact cells of photosynthetic organisms and, secondly, it enables one to eliminate the artefacts due to inactive BChl *c* molecules.

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2. EXPERIMENTAL

Chlorobium limicola strain C was grown anaerobically at 28°C. All the experiments were performed on intact 3-day-old cells in their growth medium under strictly anaerobic conditions. The 1 mm thick samples had an optical absorbance at BChl *c* absorption maximum of OD < 0.3. The fluorescence excitation spectra were recorded as well as burning was performed with a home-built CW dye laser (line width 0.5 cm^{-1}). Oxazine-1 dye was used, a Coherent CR-2000 K Kr^+-laser served as a pump. Fluorescence was recorded with a MDR-2 monochromator (dispersion 4 nm/mm) using photomultiplier RCA C31034 A-02 in photon counting regime The holes in fluorescence spectra were recorded with a DFS-24 double spectrometer (dispersion 0.45 nm/mm, typical slit widths 0.1–0.2 mm) or with a MDR-2 monochromator (slit width 0.05 mm). The spectral holes were probed by scanning the laser attenuated by a factor of 1000 over the spectral region investigated

3. RESULTS

Fig. 1 shows the fluorescence excitation and emission spectra of intact cells of *C. limicola* at 1.8K. The fluorescence excitation band with a maximum at 750 nm (at registration wavelength of BChl *a* fluorescence band maximum, 826 nm) and the fluorescence band with a maximum at ~780 nm belong to BChl *c*. In living cells of *C. limicola* the long-wave absorption maximum of BChl *c* (as well as the BChl *a* fluorescence excitation one) is well-known to vary normally between 730 and 750 nm ([7,9] and this work). However, in our experiments we failed to discover fundamental differences between cells with different positions of BChl *c* absorption maximum. Figs. 1 and 2 summarize the results of a

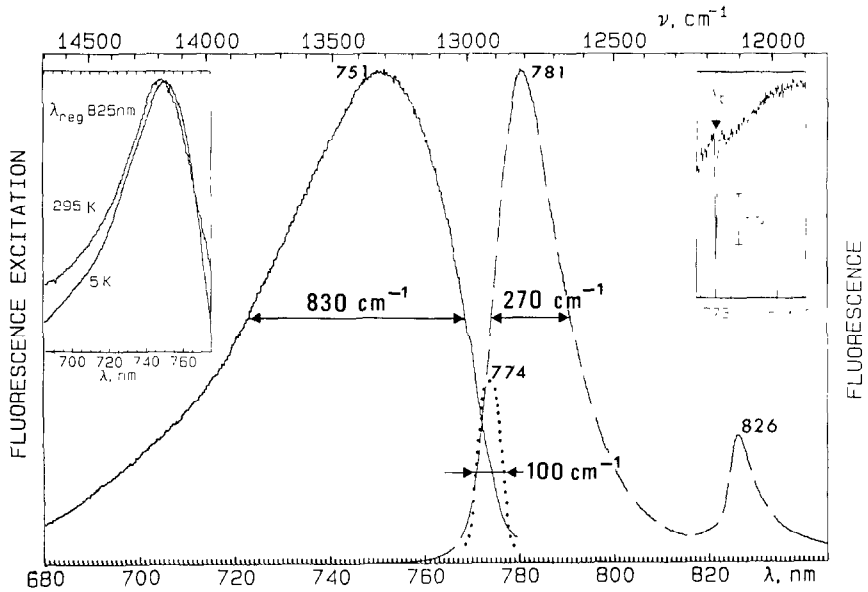

Fig. 1. The 1.8K near-infrared fluorescence excitation (solid line) and emission (dashed line) uncorrected spectra of *C. limicola* cells. Registration was at 826 nm. Excitation was at 720 nm. The dotted curve is the 1.8K spectrum of the burnt BChl *c* band on an expanded scale (see the text). (Insert to the left.) Near-infrared fluorescence excitation spectra of *C. limicola* cells at 5K and 295K. Registration was at 826 nm (Insert to the right). Difference hole-burned excitation spectrum of *C. limicola* cells at 5K. Burn conditions were: λ_b = 773 nm, 10 min at 100 mW/cm^2

whole series of experiments on the persistent hole burning in the BChl *c* band of the BChl *a* fluorescence excitation spectrum. Varying the wavelength of laser irradiation from 710 to 780 nm we have found the following.

(1) At burning wavelengths within a wide short-wave spectral range (from the short-wave edge up to the wavelength corresponding to the ~0.5-height amplitude on the long-wave slope of the excitation band), difference hole-burned excitation spectrum displays a broad non-resonant hole reproducing the contour of the excitation spectrum (Fig. 2a).

(2) At burning wavelengths within a range of the long-wave slope of the excitation band (from the wavelength corresponding to the ~0.5-height amplitude on the long-wave slope of the excitation band up to the long-wave edge of the band), difference hole-burned excitation spectrum displays both (Fig. 2b,c): (i) a sharp resonant zero-phonon hole (ZPH) and (ii) a broad non-

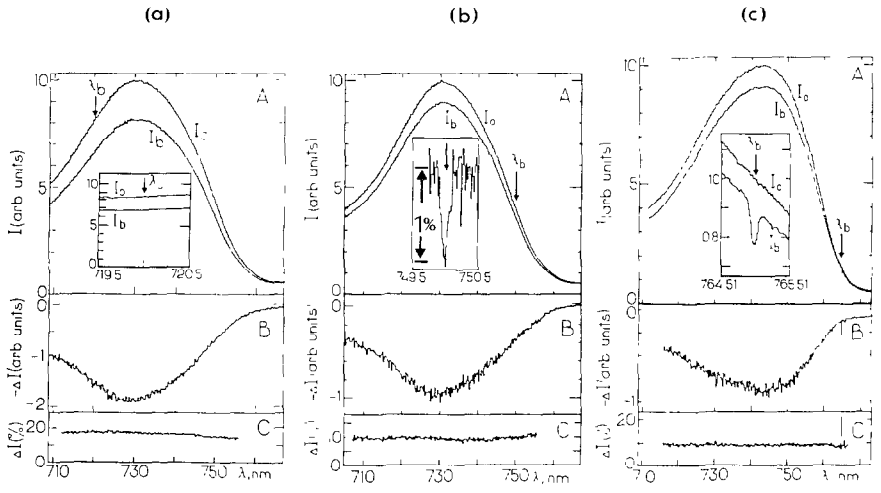

Fig. 2. The 1.8K hole-burned excitation spectra of *C. limicola* cells at (a) λ_b = 720 nm; (b) λ_b = 750 nm; (c) λ_b = 765 nm. The λ_b are shown by the arrows and the inserts represent expanded scans around these positions. (A) Excitation spectra before (I_o) and after (I_b) burning. (B) Difference hole-burned spectra (in arb. units): ΔI (a.u.) = $I_o - I_b$. (C) Difference hole-burned spectra (in %): ΔI (%) = 100 ($I_o - I_b$)/I_o. Registration was at 826 nm for all the spectra. Burn conditions were: (a) λ_b = 720 nm; 33 min at 2.5 W/cm^2; (b) λ_b = 750 nm; 16 min at 460 mW/cm^2; (c) λ_b = 765 nm; 16 min at 1.5 W/cm^2.

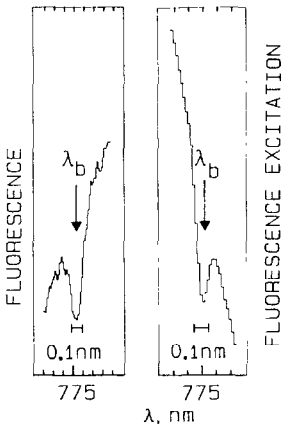

Fig. 3. The difference hole-burned spectra displaying resonant ZPH burnt simultaneously with the same λ_b in both, fluorescence and excitation spectra of *C. limicola* cells at 5K. Excitation was at 725 nm. Registration was at 825 nm. Burn conditions were: λ_b = 775.2 nm, 10 min at 7 mW/cm^2. FWHM of ZPH in fluorescence spectrum is equal to 1 Å, i.e. 1.7 cm^{-1}, uncorrected for 2 cm^{-1} read resolution. FWHM of ZPH in excitation spectrum is equal to 0.6 Å, i.e. 1 cm^{-1}, uncorrected for 0.5 cm^{-1} read resolution

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) The holes deeper than 30% are not observed.

(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 the expanded scale in Fig. 1. This lowest energy BChl *c* band has the following characteristics: (i) the bandwidth is ~100 cm^{-1}; (ii) the maximum is located at ~774 nm; (iii) at the wavelength of this maximum, the amplitude of the preburnt excitation spectrum makes up 20% of the maximum amplitude of this spectrum; (iv) the widths of ZPH (FWHM = 1 cm^{-1}, uncorrected for 0.5 cm^{-1} read resolution were found to be independent of burning wavelength but probably laser-limited. Since ZPH track λ_b, the 100 cm^{-1} FWHM of the lowest energy BChl *c* band is largely determined by inhomogeneous broadening.

(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.

4. DISCUSSION

Let us assume that indeed our antenna consists of oligomers. Then, for N molecules in each oligomer, there are N exciton states and, in principle, there are N components in the absorption (or fluorescence excitation) spectrum. Then, in 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 (the both features are found to be common for all photosynthetic antenna systems investigated [8,10–12]), one can assume for the sake of definiteness that the spectrum consists of strongly overlapping inhomogeneously broadened N bands, their widths more larger than their energy splitting with the result that the separate components of the spectrum of an oligomer cannot be resolved. Any destructive process will destroy an oligomer as a unit. In this case the spectrum of this destroyed oligomer will disappear from the common spectrum of the ensemble; instead of this N holes will be observed, one in each of N exciton bands. Then, upon burning into the lowest exciton component, only one of N holes will be resonant, and all the other $(N-1)$ higher energy holes will be non-resonant. However, if there is no correlation between the energies of N exciton states of different oligomers, then each of $(N-1)$ non-resonant holes (corresponding to the burnt $(N-1)$ higher energy exciton states) should be observed at any wavelength within its own inhomogeneously broadened exciton band. In this case, taking into account the main antenna property – the efficient excitation energy migration – one would expect that all $(N-1)$ higher energy exciton components cannot be resolved in our steady-state experiment. This means that upon burning into the lowest energy exciton component, one would expect a resonant zero-phonon hole and, simultaneously, a broad non-resonant hole reproducing the contour of the absorption (or fluorescence excitation) spectrum. Upon burning into any higher energy exciton component (non-overlapping with the lowest one), both fast downward scattering to any lowest energy level within the lowest inhomogeneous exciton band (again due to lack of correlation between the 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 should observe only a broad hole reproducing the contour of the absorption (or fluorescence excitation) spectrum of such a system.

In the opposite case of weak interactions between all the antenna molecules, i.e. if these N bands of the spectrum are due to uncoupled antenna pigment monomers, only one resonant hole (due to zero-phonon line) in the lowest energy band would be observed upon burning into it since there is no uphill energy transfer at helium temperatures.

Thus, the persistent hole spectra described in section 3 are consistent with an excitonically coupled BChl *c* chromophore system if all the assumptions made are valid. Let us examine them.

First, the presence of several bands in the spectrum (as well as their excitonic nature) is demonstrated by the linear [9] and circular [13] dichroism spectra, by the fourth derivative absorption spectra of living cells [14],

and, at last, direct separation of the 0–0 band of the lowest exciton component, 774 nm band (this work). If the near-infrared BChl *c* band is 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 experiments will not alter.

Second, our conclusion is valid within the limits of weak exciton–phonon coupling. For type 774 nm band, the weak coupling is reflected in the small intensity of the pseudo-phonon sideband (Fig. 1). Besides, at 5K the width of the BChl *c* band is about 90% of its room-temperature value (Fig. 1). Such a small change in the width suggests that a contribution of phonon sidebands and low frequency vibrations to the BChl *c* band contour is rather small.

Third, we assume that the excited states of BChl *c* chromophores are inhomogeneously broadened. For the 774 nm band, the magnitude of inhomogeneous broadening was shown to be equal to 100 cm^{-1}. As to the higher energy exciton components, it should be mentioned that our fundamental conclusion is independent of their bands being homogeneously or inhomogeneously broadened.

Fourth, we assume that there is no correlation between the energies of exciton states of different BChl *c* oligomers. This assumption has no direct experimental proof yet but the same type of assumption allowed an understanding of a number of the observed spectral features in other natural antenna systems [10,15].

Thus, the hole spectra measured for *C. limicola* cells were shown to be consistent with the BChl *c* organization in chlorosomes as BChl *c* oligomers.

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