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Hole-burning study of exciton migration and pigment–protein interaction in photosynthetic systems

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Abstract

Spectral hole burning enabled to determine excited state lifetimes T_1 calculated from widths δ_{ZPH} of resonant zero phonon holes. Values of δ_{ZPH} reflected ultra-fast exciton migration within the photosystem II reaction centers containing (5 and 6) chlorophylls *a*, (2) pheophytines *a* and (1) β-carotene. This technique moreover provided Debye–Waller factor α and mean frequency of protein phonons Ω, which characterized the pigment–protein interactions. The resonant holes were observed together with their vibronic satellites burnt both into absorption and fluorescence spectra of the reaction centers. The phenomenon of laser induced hole-filling of the primary hole after the burning of the secondary holes at different wavelengths, was used to study the role of fast de-excitation processes in hole burning.

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Keywords: Hole burning; Exciton and electron migration; Primary photosynthetic events; Reaction center of photosystem II

1. Introduction

Photosynthesis in green plants and cyanobacteria occurs mainly in photosystem two (PS II) and in photosystem one (PS I) embedded in a thylakoid membrane. The connection of PS II and PS I enables the production of a very high oxidizing potential (≈ 1 V) which is used for water oxidation accompanied by oxygen evolution. This is a major difference between bacterial and plant photosynthesis.

PS II is that part of the photosynthetic apparatus that oxidizes water to produce the hydrogen equivalents required to reduce carbon dioxide to organic substances. Dioxygen is the by-product of this light-driven process. The reaction center of PS II consisting of the D1 and D2 proteins, the α and β subunits of cytochrome *b*559 (Cyt *b*559) and the product of the *psbI* gene was first isolated in Ref. [1]. Since that time the PS II reaction center (RC) complexes, containing a well-defined number of chlorophylls *a* (5 and 6), pheophytines *a* (2) and β-carotenes (1 and 2) were prepared using various biochemical techniques [2].

Primary processes in photosynthesis occur within a thylakoid membrane. It involves the

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absorption of light by antenna complexes and the excited energy transfer (EET) to a primary electron donor within a reaction center where the energy is trapped by a sequence of electron-transport (e.t.) reactions. Sufficient resolution of X-ray diffraction and electron microscope structural methods is up to now achieved only for RC and antenna of purple bacteria. The molecular structure is not well known in PS II RC and in most of their antennae. The understanding and interpretation of EET in these systems are therefore much more complicated and must be based mainly on the application of high-resolved spectroscopic studies of their functional properties. Fast (fs–ps) EET and e.t. processes are widely studied directly by means of time-resolved absorption and fluorescence spectroscopy as well as indirectly using hole-burning technique.

The PS II RC prepared with various numbers of photosynthetic pigments was studied by means of hole-burning spectroscopy by several authors. The first attempt was done in Ref. [3]. Narrow resonant zero-phonon holes ($\delta_{ZPH} \approx 1\ cm^{-1}$) were observed together with off-resonant broad holes ($\delta \approx 120\ cm^{-1}$) in absorption spectra of PS II RC prepared by various isolations (≈ 10 Chl *a*). Later [4], the same technique was used to study excited energy transfer from the nearest CP47 antenna complex to the RC. Hole-burning effects were also observed in fluorescence spectra of PS II particles in Ref. [5]. The investigation of vibronic satellite side-band holes moreover provided Debye–Waller factor α and mean frequency of protein phonons Ω to characterize the pigment–protein interactions in Refs. [6,7]. Systematic hole-burning study was performed mainly in fluorescence excitation and partially in absorption spectra of PS II RC containing 5 and 6 Chl *a* in Ref. [8]. Very high resolution and sensitivity (together with 10^6–10^6 s, transient technique) enabled the resolution of very narrow holes of trap pigments from the background of $\approx 1\ cm^{-1}$ broad resonant holes. The obtained effective homogeneous line width $\Gamma_{hom} = (2\pi \boldsymbol{T}_1)^{-1} = (40 \pm 10)$ MHz for $\boldsymbol{T} \to 0$ K corresponds to the fluorescence lifetime (4 ± 1) ns of Chl *a* (not influenced by fast EET) which acts as a trap [9].

In this contribution we focus to the exciton migration between particular pigments and the role of pigment–protein interaction studied by means of low-temperature absorption, fluorescence and both resonant and off-resonant hole-burning spectroscopy. Moreover, the laser-induced hole-filling (LIHF) of primary hole after the burning of a secondary hole at different wavelengths was used to study hole-burning mechanisms of fast de-excitation processes.

2. Materials and methods

PS II RC complexes were prepared by using immobilized metal affinity chromatography [2]. These PS II RC complexes contain well-defined number of pigments: 5 or 6 chlorophylls *a* (Chl *a*), 2 pheophytines *a* (Pheo *a*) and 1 β carotenoide (β Car). The PS II RC complexes were dissolved in 60% glycerol buffer and slowly frozen in a bath or gas-flow cryostat to produce transparent samples at low temperatures (4.2–25 K).

Persistent hole-burning experiments were performed in both absorption and fluorescence spectra of PS II RC containing 5 and 6 Chl *a*. Fluorescence was excited in Soret absorption of chlorophylls and pheophytines using blue and green lines of cw Ar-ion laser. Emitted light was analyzed in double-grating monochromator (with a resolution of $0.5\ cm^{-1}$). Absorption spectra were measured in single-channel mode by means of stabilized tungsten lamp together with double-grating monochromator (resolution $0.5\ cm^{-1}$, sensitivity ± 0.001 OD). Both fluorescence and absorption signals were detected in cooled photomultipliers along with a lock-in amplifier. The holes were burnt both into Q_y absorption band and into the main fluorescence band by cw dye lasers (Spectra Physics 375 and 380). Zero-extrapolated value of zero phonon hole width was determined from intensity and burning time fluence curves to remove a power broadening.

Laser induced hole-filling experiments were performed similarly as described in Refs. [10,11]. Primary hole was burnt by the cw dye laser tuned at the primary wavelength λ_B. Then the dye laser was tuned to the secondary burning wavelength $\lambda_S \neq \lambda_B$ and the whole procedure of hole-burning while slowly increasing the power (***P***) and the burning time (***t***) was repeated. The relative hole-filled area (***A***)

increased with increase of the secondary burning exposition $A = A(P, t)$. The mathematical description of the function $A(P, t)$ was systematically studied for various λ_S. The hole-burning and de-excitation mechanisms were discussed in the frame of observed LIHF trends.

3. Results and discussion

Fig. 1 shows typical low-temperature absorption and fluorescence spectra of the PS II RC containing 6 or 5 Chl *a*, 2 Pheo *a* and 1 β Car molecules. The absorption bands at 417 and 434 nm are well resolved at low temperatures in the PS II RC containing 6 Chl *a*, 2 Pheo *a* and 1 β Car molecules in comparison with those in the PS II RC containing 5 Chl *a*, 2 Pheo *a* and 1 β Car molecules. Molecules of β Car absorb mainly in three bands at 459, 488 and 505 nm. The Q_x absorption of Chl *a* and Pheo *a* can be well resolved at 543 nm and 629 nm, respectively. On the other hand, the Q_y absorption bands of Chl *a* and Pheo *a* are mixed together between 660 and 690 nm. The low-temperature fluorescence spectra consist of an intensive emission band at 685 nm which corresponds to an emission of a trap in PS II RC. The fluorescence spectra are identical for all excitations in absorption bands of Chl *a*, β Car and Pheo *a* (457.8, 488.0, 514.5 nm). This is caused by fast vibronic relaxation from higher singlet states of Chl *a*, and Pheo *a*. The excitation is effectively transferred from the second singlet states of β Car to the Chl *a*, and Pheo *a* in PS II RC.

Fig. 2 shows a typical resonant zero-phonon hole together with its vibronic satellites burnt into the absorption and fluorescence spectra. Widths of the observed zero-phonon holes follow the expression $\delta_{ZPH}(Pt) = \delta_{ZPH}(0) + a[Pt]^b$. Zero-extrapolated value of zero-phonon hole width ($4.2\,cm^{-1} \leq \delta_{ZPH}(0) \leq 5.1\,cm^{-1}$) corresponds to excited lifetimes (T_1 pure relaxation time ($2.1\,ps \leq T_1 \leq 2.5\,ps$). These values are in good agreement with widely accepted rate constants of exciton migration in PS II RC. Proportional coefficients of power broadening ($a \approx 0.74$) were the same in absorption hole-burning for PS II RC containing both 5 and 6 Chl *a*. The parameter b ($0.23 \leq b \leq 0.26$) is close to 1/4 which according to Ref. [12] corresponds to dispersive kinetics of hole burning of the PS II RC.

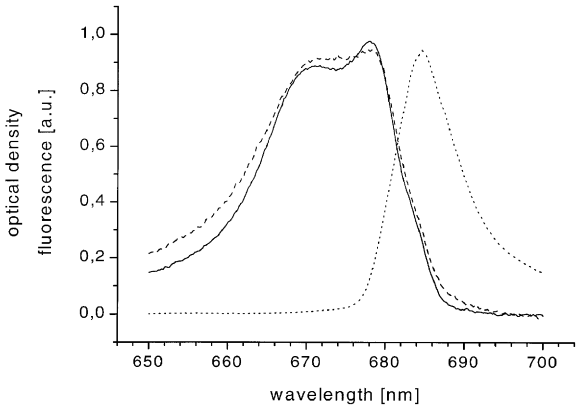

Fig. 1. Resonant low-temperature absorption and fluorescence (dotted) spectra of the PS II RC containing 6 Chl *a* (dashed) or 5 Chl *a* (full), 2 Pheo *a* and 1 β Car molecules.

The vibronic satellite side-band hole is blue shifted ($20\,cm^{-1}$), while pseudo-phonon hole is red shifted ($15\,cm^{-1}$) with respect to the zero-phonon hole in the absorption spectra. On the other hand both satellite side-band hole and pseudo-phonon hole are red shifted ($30\,cm^{-1}$), with respect to the zero-phonon hole in the fluorescence spectra. The Stokes shift (2Δ) was found to be $40\,cm^{-1}$ in PS II RC 5 : 1 and $42\,cm^{-1}$ in PS II RC 6 : 1. The obtained mean frequency of pigment–protein phonons was $\Omega = 50\,cm^{-1}$ in PS II RC 5 : 1 and $48\,cm^{-1}$ in PS II RC 6 : 1. The Debye–Waller factor ($\alpha = 0.7 \pm 0.1$) corresponds to pigment–protein interaction in RC.

LIHF results enabled the determination of the monoexponential decay of the area (A) of the filled primary hole for $\lambda_S > \lambda_B$. The parameter τ_R of the dependence $A = A_{as} + A_0 \exp(-Pt/\tau_R)$ was $\approx$ 10 J. The LIHF dependence is shown in Fig. 3. The LIHF dependence for $\lambda_S < \lambda_B$ (not shown) exhibits biexponential decay with parameters τ_R and τ_{EET}. The parameter τ_R is the same as above, while $\tau_{EET} \approx 1$ J.

The more effective biexponential LIHF decay for $\lambda_S < \lambda_B$ is caused by at least two filling mechanisms. The first filling mechanism is photoinduced relaxation of the protein matrix. This photoinduced

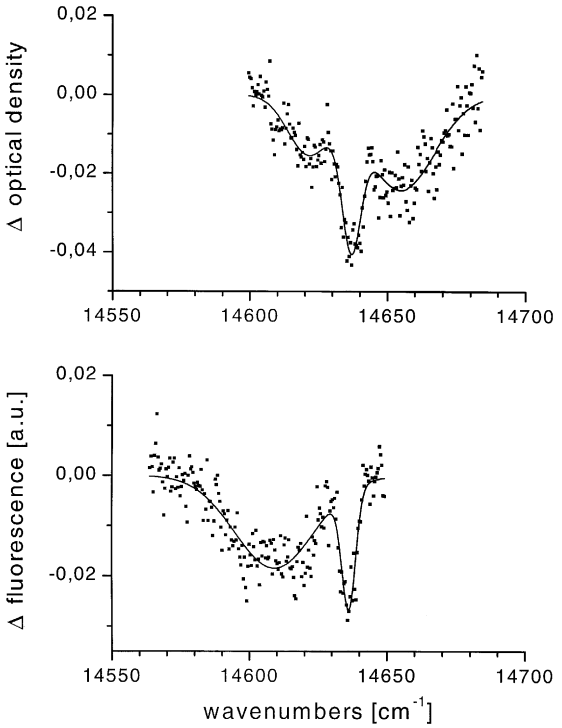

Fig. 2. Resonant zero-phonon holes together with their vibronic satellites burnt into absorption (above) and fluorescence (below) spectra.

relaxation ($\tau_R \approx 10$ J) occurs for all λ_S in the Q_y absorption band. The second LIHF mechanism is due to backward photoconversion of the antihole sites. These antihole sites are excited indirectly by means of fast EET from the sites absorbing at λ_S. Our LIHF results obtained in PS II RC 5 : 1 are in good agreement with LIHF trends observed in core antenna of PS II [10].

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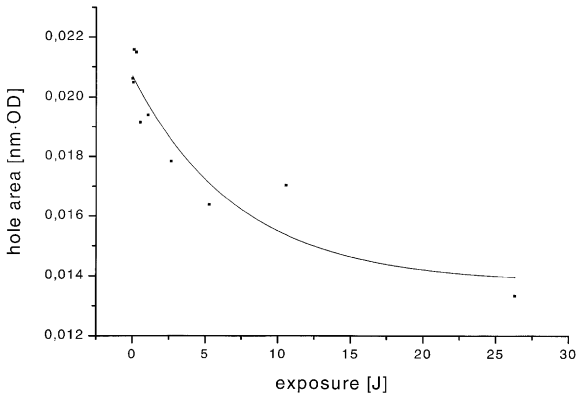

Fig. 3. Laser-induced hole filling of the primary burnt hole $\lambda_S > \lambda_B$.

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