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Low-temperature absorption off-resonant hole-burning spectroscopy of pheophytine *a* in photosystem II reaction center

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Abstract

Chlorophylls, pheophytines and carotenes are main photosynthetic pigments. They play a crucial role in primary photosynthetic processes (excited energy transfer and electron transport). In this contribution we report on both resonant and off-resonant hole-burning low-temperature absorption spectra. The resonant narrow zero-phonon holes together with broad ($\approx 84\,\mathrm{cm}^{-1}$) off-resonant holes were burnt into Q_y absorption bands, while very broad ($\approx 250\,\mathrm{cm}^{-1}$) off-resonant satellite holes were observed in Q_x absorption transitions. This technique enabled one to distinguish between satellite holes of chlorophyll *a* and pheophytine *a* and consequently to localize spectral bands of both pheophytines *a* in the complex Q_y absorption spectrum of reaction center of photosystem II containing six chlorophylls *a*, two pheophytines *a* and one β-carotene. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Primary photosynthetic events; Reaction center of photosystem II; Pheophytine *a*; Off-resonant spectral hole burning

1. Introduction

The role of both molecules of pheophytine *a* (Pheo *a*) in reaction center (RC) of photosystem II (PS II) is still a matter of debate. 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. Oxygen is the by-product of this light-driven process. A RC of PS II consisting of the D1 and D2 proteins, the α and β subunits of cytochrome b559 and the product of the psbI gene was first isolated in Ref. [1]. Since then the PS II RC complexes containing well-defined number of five and six chlorophylls *a* (Chl *a*), two Pheo *a* and one and two β-carotenes (β-Car) were prepared using various biochemical techniques [2]. One molecule of Pheo *a* is believed to take part in electron transport (e.t.) as primary electron acceptor. The excited special Chl *a* pair in RC (primary donor in RC) generates an unpaired electron which is transferred within several picoseconds towards the Pheo *a* acceptor. Later (after ≈ 100 ps) a sequence of slower e.t. reactions follows. On the other hand the role of the second molecule of Pheo *a* in RC is not clear. The second molecule of Pheo *a* does not take part in e.t., while its participation in excited energy transfer (EET) towards special pair in RC (primary donor in RC) is not excluded. An alternative interpretation of primary donor is based on a multimer model in which both special Chl *a* pair and the Pheo *a* acceptor are included [3]. 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 employing hole-burning technique. The attempts to calculate the PS II RC structure from homology of D1, D2 proteins with L and M proteins in purple bacteria RC were published in Ref. [4]. The conformation of the Chl *a* and Pheo *a* cofactors in the model was optimized by using exciton interaction calculations in combination with potential energy calculations. The primary donor P680 is proposed to be a weakly coupled Chl *a* dimer, while the primary acceptor is Pheo *a* monomer.

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Spectral behavior of Pheo *a* in PS II RC was studied by means of time-resolved spectroscopy by several authors. Neither femtosecond transient absorption measurements [5] nor picosecond transient fluorescence measurements [6] of PS II solved definitively this problem. Site selection [7] cw measurements offered a decomposition of RC PS II absorption spectrum obtained at 10 K into 9 bands. They assigned the most pronounced band to the absorption of the primary electron donor (P680). Six medium absorption bands were assigned to three couples of Pheo *a*, accessory, and antenna Chl *a*. Furthermore, three weak (minor) bands were interpreted as absorption bands of three additional Chl *a*.

The first hole-burning attempt was done in Refs. [8,9]. Narrow resonant zero phonon holes ($\delta_{\mathrm{ZPH}} \approx 1\,\mathrm{cm}^{-1}$) were observed together with broad ($\delta \approx 120\,\mathrm{cm}^{-1}$) off-resonant holes in Q_y absorption spectra of PS II RC prepared by various isolations (≈ 10 Chl *a*). Hole-burning effects were also observed in fluorescence spectra of PS II particles in [10,11]. Systematic resonant 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 [12,13] including a pilot experiment in off-resonant hole burning into Q_x band of Pheo *a*. High resolution and sensitivity (together with 10^{-6}–10^{6} s, transient technique) enabled to resolve very narrow holes of trap pigments from the background of $\approx 1\,\mathrm{cm}^{-1}$ broad resonant holes. The obtained effective homogeneous line width $\Gamma'_{\mathrm{hom}} = (2\pi T_1)^{-1} = (40 \pm 10)$ MHz for $T \to 0$ K corresponds to the fluorescence lifetime (4 ± 1) ns of Chl *a* and Pheo *a* (not influenced by fast EET) which acts as a trap [14,15].

The aim of this contribution is to determine the Q_y absorption profiles of both Pheo *a* in the Q_y absorption spectrum of the whole RC by means of systematic Q_x absorption off-resonant hole measurements.

2. Materials and methods

PS II RC complexes were isolated from *Pisum sativum* according to Ref. [2] with slight modifications. Q Sepharose was used instead of immobilized metal affinity chromatography. A column containing PS II RC complexes was washed with 20 mM phosphate buffer containing 0.02% dodecylmaltoside (DM) and reaction centers were eluted from the column with gradient elution up to 200 mM $MgSO_4$ in 20 mM phosphate buffer and 0.02% DM. Those complexes contain well-defined number of 6 Chl *a*, 2 Pheo *a* and 1 β Car. For low-temperature spectroscopy the PS II RC complexes were dissolved in 60% glycerol–buffer and slowly frozen in a bath or a gas-flow cryostat to produce transparent samples.

Absorption spectra were measured in single-channel mode by means of stabilized 250 W tungsten lamp together with double grating monochromator (resolution $0.5\,\mathrm{cm}^{-1}$, sensitivity ±0.001 OD). The absorption signals were detected in cooled photomultipliers along with a lock-in amplifier. The holes were systematically burnt into whole Q_y absorption band by cw dye laser (Spectra Physics 375, equipped with 3-plate birefringent filter and thin etalon). The spectral width of the laser was $0.3\,\mathrm{cm}^{-1}$. The laser power was $200\,\mathrm{mW cm}^{-2}$, the exposition ranged from 0 to 1800 s. Persistent absorption holes were detected both resonant and off-resonant in Q_y band of PS II RC complex and off-resonant in Q_x band of Pheo *a*.

3. Results and discussion

Figs. 1 and 2 show low-temperature absorption resonant holes of PS II RC complex and off-resonant hole of Pheo *a*, respectively. The holes observed in Q_y absorption of PS II RC consist of a resonant narrow zero-phonon hole (ZPH) together with a $84\,\mathrm{cm}^{-1}$ broad off-resonant hole. The spectral position of the ZPH changed as the dye laser was tuned, while the broad off-resonant hole was always at the same spectral position (around 681 nm.). The total area of the Q_y hole after 1800 s of the burning reached the value 0.3 OD nm for the burning wavelengths around 681 nm. On the other hand the total area of the Q_y hole was less than one order below this value at the edges of the absorption band of the PS II RC. The behavior of the resonant holes is in good agreement with that observed in Refs. [8,9]. However in the Q_y off-resonant hole-burning experiments we obtained the value $84\,\mathrm{cm}^{-1}$ instead of that ($120\,\mathrm{cm}^{-1}$) published in

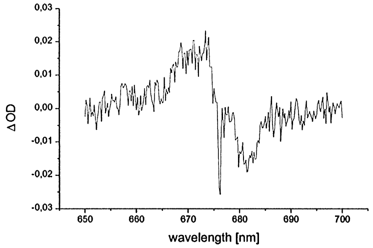

Fig. 1. Typical resonant hole burnt and detected in Q_y absorption band of RC PS II containing 6 Chl *a*, 2 Pheo *a* and 1 *β* Car. The current burning wavelength was 6761 nm.

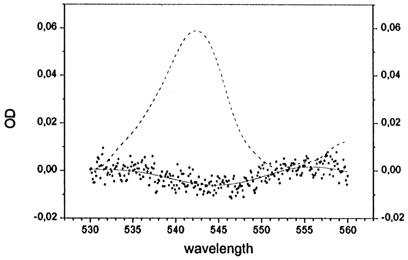

Fig. 2. Off-resonant hole (solid) detected in Q_x absorption band (dashed) of 2 Pheo *a*. The burning was performed into Q_y absorption band of RC PS II containing 6 Chl *a*, 2 Pheo *a* and 1 β Car between 650 and 700 nm.

Ref. [8]. We believe that this difference is due to our better preparation of PS II RC. Moreover we systematically burnt and measured in the broader spectral region.

In the Q_x absorption of Pheo *a* we observed very broad ($\approx 250\,\mathrm{cm}^{-1}$) off-resonant holes around 546 nm. This hole width fits well with the width of Q_x absorption band. The area of the Q_x off-resonant hole increases with exposition at all burning wavelengths within the Q_y absorption band of PS II RC. The maximum hole area was below 0.5 OD nm. These values were plotted for the expositions 300, 900, 1800 s against the burning wavelengths and fitted by two Gaussian curves. These Gaussian curves are shown together with Q_y absorption spectrum of the of PS II RC in Fig. 3.

Our results show that there are two spectrally different forms of Pheo *a* in RC PS II complex. We interpret these Gaussian curves as absorption bands of particular Pheo *a*. The less-pronounced blue-shifted absorption Q_y band of Pheo *a* at 670 nm, (FWHM = 10 nm) exhibits a weaker hole-burning efficiency. This is due to an efficient EET from this Pheo *a* towards primary donor and/or the trap. On the other hand the more pronounced, red-shifted absorption Q_y band of Pheo *a* at 681 nm, (FWHM = 11 nm) was saturated even after 300 s of burning (see Fig. 4). This higher hole-burning efficiency can be connected with involving this Pheo *a* molecule in primary photosynthetic processes. The spectral position 681 nm is a little red shifted with respect to the 679 nm determined in Ref. [7]. Konermann et al. suggested in Ref. [7] the decomposition with the same absorption strengths of both Pheo *a*, which seems to be unlikely due to the significantly different role of both the Pheo *a* molecules in PS II RC. The fact that both Pheo *a* Gaussian profiles exhibit within our accuracy the same FWHM excludes any EET (e.g. from Chl *a* molecules burnt in Q_y band) towards these Pheo *a* molecules. If there would be such a EET, the Pheo *a* could also be burnt via this EET and we would observe not only off-resonant hole-burning contribution from Pheo *a* but also additional off-resonant hole-burning contributions from other (Chl *a*) molecules and consequently more complex profile than two Gaussian curves of the same FWHM. Our off-resonant hole-burning results exclude the presence of Pheo *a* from the multimer model of PS II RC [3] at low temperatures.

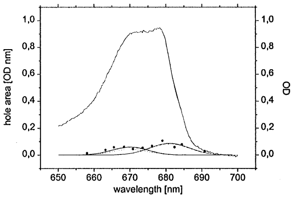

Fig. 3. Low-temperature absorption Q_y of the RC PS II containing of 6 Chl *a*, 2 Pheo *a* and 1 β Car molecules together with 2 Gaussian profiles of 2 Pheo *a* determined from off-resonant hole-burning area in Pheo-*a* Q_x after 300 (dotted), 900 (dashed) and 1800 (solid) seconds of burning (200 mWcm^{-2}). The experimental data points of the area of the Q_x hole are denoted by solid circles.

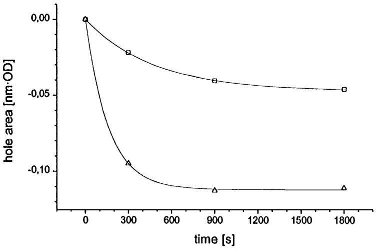

Fig. 4. Dependence of off-resonant burnt hole area on burning time for different burning wavelength. The squares represent the dependence in the blue region of Q_y (658, 663, 665 nm), triangles in red region of the Q_y (679 nm).

Acknowledgements

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