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Low temperature absorption, fluorescence, and hole-burning spectroscopy of photosystem II reaction center complex containing 1 and 2 carotenoides

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Abstract

Well defined photosystem II reaction centers from *Pisum sativum* containing 5 or 6 chlorophyll a (Chl a), 2 pheophytine a (Pheo a), and 1 or 2 β-carotene (β Car) molecules were prepared by using immobilized metal affinity chromatography. Samples containing 6 Chl a and 1 or 2 β-Car and containing 5 Chl a and 1 β-Car were measured using low temperature absorption, fluorescence and hole-burning spectroscopy.

Absorption bands of the β Car (462, 490, and 508 nm) can be clearly distinguished next to the Soret absorption band of Chl a at low temperature. Their relative intensities strongly depend on Chl/Car ratio. The shapes of fluorescence bands are the same for all samples.

Persistent spectral holes were burnt into both absorption and fluorescence spectra. This technique provides lifetime of excited state τ_1 and Huang–Rhys factor *S*. Values of τ_1 correspond to two picoseconds energy transfer in reaction centers. Huang–Rhys factor $S = 0.4$ appears to be the same for all studied samples. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Hole-burning; Optical spectroscopy; Carotenoides; Photosystem II reaction center

1. Introduction

Photosynthetic reactions in green plants occur in thylacoid membranes of chloroplasts. Primary photosynthetic reaction – charge separation – takes place in reaction centers. There are two photosystems in green plants: photosystem II, and photosystem I. Ultrafast kinetics of primary photosynthetic processes can be studied directly by means of time-resolved optical spectroscopy and indirectly using spectral hole-burning technique [1].

Hole-burning spectroscopy is a unique method which determines the homogeneous width of optical transitions. It is possible to determine from the hole burned into spectrum not only the homogeneous linewidth (and therefore the lifetime of excited state), but also the strength of pigment–protein interaction. Detailed description of the hole-burning spectroscopy is provided for instance in [2].

2. Experimental

The photosystem II reaction center samples were prepared from *Pisum sativum* by using immobilized

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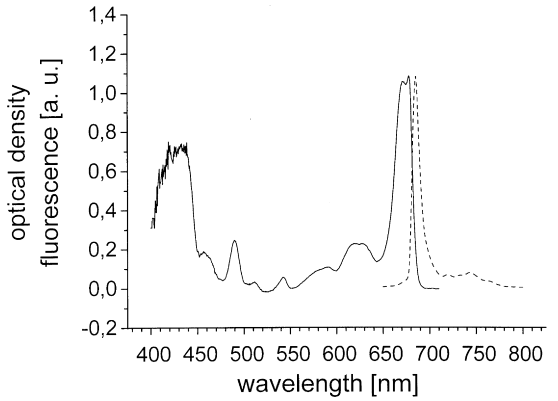

Fig. 1. Absorption (solid line) and fluorescence (dashed line) spectra of the photosystem II reaction center containing 6 Chl a and 1 β Car measured at 6 K.

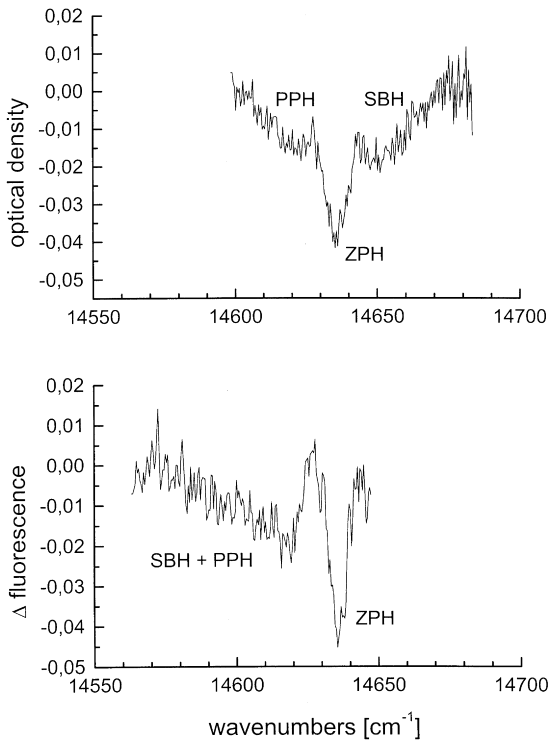

Fig. 2. Resonant zero phonon holes together with their vibronic satellites in absorption (top) and fluorescence (bottom) spectra of the photosystem II reaction center containing 6 Chl a and 1 β Car. ZPH: zero phonon hole, SBH: phonon sideband hole, PPH: pseudo phonon hole.

metal affinity chromatography as described in [3]. There were prepared three samples that differ at Chl a/β Car ratio: 6/2, 6/1, and 5/1. Special low temperature measuring cells were filled in dark at 0°C. Between experiments the cells with samples were stored in liquid nitrogen.

All experiments were carried out using our home-build experimental setup. Samples were kept on desired temperature in continuous flow cryostat Oxford Instruments CF 1204. Dye laser Spectra Physics 375B with pyridine (Lambda Physics) pumped by argon ion laser ILA 190 (Carl Zeiss) provided burning light of spectral width about 0.3 cm^{-1}. Wavelength of burning light was determined using Jobin-Yvon HRD1 monochromator and multichannel analyzer OVA 284.

For measurements of absorption spectra was used 500 W tungsten lamp as a source of light. Its light was monochromated by two gratings monochromator Jobin-Yvon HRD1, minimal spectral width 0.6 Å. Monochromator output was chopped and focused onto the sample. Transmitted light was detected by RCA C31034A photomultiplier and amplified by a lock-in amplifier. Photomultiplier tube was cooled by mixture of ethanol and liquid nitrogen.

The ILA 190 laser provided both pumping of dye laser and excitation light in fluorescence setup. Excitation beam was chopped, desired wavelength of excitation was chosen by interference filters. Both excitating and burning beams were focused to sample in cryostat using reflecting prisms. Fluorescence of sample was collected by two lens onto input of HRD1 monochromator. Monochromator output was detected by Hamamatsu R943-02 photomultiplier cooled by Hamamatsu Peltier-cooler C2761. Signal was amplified by the lock-in amplifier.

3. Results

Absorption and fluorescence spectra of the sample containing 6 Chl a/1 β Car measured at 6 K are shown at Fig. 1. All three bands of β Car (462, 490, and 508 nm) can be at this temperature easily distinguished next to Soret band of Chl a. Wavelength of their maxima is the same in absorption spectra of all three materials, while their relative intensity strongly depends on the Chl a/β Car ratio. The peak at 543 nm

belongs to Q_x transition of Pheo a and it is independent on Chl a/β Car ratio.

Fluorescence spectra of all three materials have the same shape, wavelength of their maxima slightly changes (for samples with Chl a/β Car ratio of 6/2, 6/1, and 5/1 these are 684.3, 684.6, and 683.8 nm respectively). The shape of the fluorescence bands is identical for both excitating wavelengths 488.0 and 514.5 nm using 40 mW power. Fluorescence excited in the second vibrational band of β Car (488.0 nm line) is weaker than that excited in the low energy vibrational band of β Car (514.5 nm line).

Spectral holes were burnt into both absorption and fluorescence spectra of all three materials. Fig. 2 shows the spectral holes burnt at 683.30 nm into absorption and fluorescence spectra of the sample containing 6 Chl a and 1 β Car. The vibronic phonon sideband hole (SBH) is blue shifted while pseudo phonon hole (PPH) is red shifted in respect to the zero phonon hole (ZPH) in the absorption spectrum. In contrast, the PSH and the PPH are overlapped at the low-frequency side of the ZPH in the fluorescence spectrum. The antiholes are situated on both sides of the ZPH.

The holewidth in absorption of the sample containing 6 Chl a and 2 β Car is 4.9 cm^{-1} (corresponding lifetime is 2.2 ps) and in its fluorescence 5.2 cm^{-1} (lifetime 2.0 ps). The similar results were obtained for the sample containing 6 Chl a and 1 β Car: in absorption 4.7 cm^{-1} and in fluorescence 5.1 cm^{-1}. Corresponding lifetimes are 2.3 and 2.1 ps, respectively. Hole in absorption spectrum of sample containing 5 Chl a and 1 β Car has width 5.2 cm^{-1} (lifetime 2.0 ps), in fluorescence 4.2 cm^{-1} (lifetime 2.5 ps). Huang–Rhys factors S were determined from the ratio of the PSH and ZPH in absorption spectra. The values of S ranged around 0.4 for all three systems.

4. Discussion

The ratio of intensities of absorption bands of Chl a, Pheo a, and β Car of all three materials well corresponds to the stoichiometry of pigments in reaction centers. Relative intensities of the three vibronic absorption bands of β carotene strongly depend on the Chl a/β Car ratio. Possible interpretation is based on conformation changes in reaction center caused by removing of particular pigments. No change in wavelength of maxima of these vibronic absorption bands was observed.

The similarity of shapes of fluorescence spectra of all three samples shows that in all measured materials is the light emitted by the same luminophore. Fluorescence excited at 514.5 nm (at the low energy vibronic absorption band) is stronger than that excited at 488.0 nm (the second vibronic absorption band) using the same excitation power. Therefore the excited energy is transferred from β carotene faster than the vibrational relaxation. It corresponds to very short lifetime of the second excited state of β carotene [4]. The excited energy is transferred from higher vibrational state of β carotene to another acceptor than from low vibrational state. The shape of fluorescence bands is the same for both excitating wavelengths and thus the excited energy is finally transferred to the same luminophore, however the excited energy transfer path from second vibrational state has lower quantum efficiency.

The results of hole-burning experiments demonstrate about two picosecond excited energy transfer within reaction centers of photosystem II. The variation of number of β Car molecules does not change the time constant of the excited energy transfer. However, when one molecule of Chl a is removed, the ZPH width in fluorescence decreases about 20% while the ZPH width in absorption remains unchanged. The strength of pigment–protein interaction determined by the Huang–Rhys factor remains in all three samples unchanged. The difference in ZPH widths measured in absorption and fluorescence is explained as follows: All molecules that absorb at the burning wavelength are excited during hole burning procedure. ZPH detected in absorption corresponds to a drop of absorption of all these molecules. However, ZPH observed in fluorescence originated from a decrease of molecules of the luminophore only. In this context the removing of one Chl a from reaction center causes longer lifetime of exciton in the luminophore.

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