Photoinduced changes in photosystem II pigments

Atanaska S Andreeva¹, Mira C Busheva², Katerina V Stoitchkova¹,³ and Iren K Tzonova²

¹Sofia University, Faculty of Physics, Department of Condensed Matter Physics, 5, J. Bourchier blvd., 1164 Sofia, Bulgaria

²Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev str. bl.21, 1113 Sofia, Bulgaria

E-mail: katys@phys.uni-sofia.bg

Abstract The photosynthetic apparatus in higher plants performs two seemingly opposing tasks: efficient harvest of sunlight, but also rapid and harmless dissipation of excess light energy as heat to avoid deleterious photodamage. In order to study this process in pigment-protein supercomplexes of photosystem II (PSII), 77 K fluorescence and room temperature (RT) resonance Raman (RR) spectroscopy were applied to investigate the changes in structure and spectral properties of the pigments in spinach PSII membranes. The high-light treatment results in a strong quenching of the fluorescence (being largest when the excitation is absorbed by carotenoids) and a red-shift of the main maximum. Decomposition of the fluorescence spectra into four bands revealed intensive quenching of F685 and F695 bands, possible bleaching of chlorophyll a, enhanced extent of light harvesting complexes (LHCII) aggregation and increased energy transfer to aggregated LHCI I. The analysis of RR spectra revealed the predominant contribution of ß-carotene (ß-Car) upon 457.8 and 488 nm excitations and lutein (Lut) at 514.5 nm. During prolonged exposure to strong light no significant bleaching of ß-Car and weak photobleaching of Lut is observed. The results will contribute to the efforts to produce more efficient and robust solar cells when exposed to fluctuations in light intensity.

1. Introduction

Pigments in plants absorb almost 100% of the sunlight reaching them and transform it into other forms of energy. Using this energy the membrane bound plant pigment-protein complex of photosystem II catalyses the splitting of water into molecular oxygen via the so-called "oxygen-evolving complex" (OEC) [1-3]. PSII is surrounded by a number of trimeric light harvesting chlorophyll (Chl) a/b protein complexes II and some minor ones, forming the PSII–LHCII supercomplex [4]. PSII comprises core antenna proteins CP47 and CP43, reaction center subunits D1 and D2, three membrane-extrinsic subunits forming OEC and many small proteins. It binds 35 Chl a molecules and 12 ß-Car molecules [4,5].

The photosynthetic apparatus in higher plants performs two seemingly opposing tasks: efficient harvesting of sunlight, but also rapid and harmless dissipation of excess light energy as heat to avoid deleterious photodamage under high light intensities. In order to study this process in pigment-protein supercomplexes of PSII, 77 K steady-state emission fluorescence and room temperature (RT)
resonance Raman spectroscopy were applied to investigate the changes in structure and spectral properties of the major pigments in spinach PSII membrane preparations due to treatment with strong light intensity.

2. Materials and methods

2.1. Isolation of PSII submembrane particles

PSII enriched subchloroplast fraction (BBY) was isolated from market spinach following the modified procedure [6]. BBY particles were resuspended in media containing 20mM Hepes (pH 7.5), 15 mM NaCl, 5 mM MgCl$_2$, 0.4 M sucrose and stored at -20°C. The chlorophyll content of the samples was estimated by using the method of Lichtenthaler [7].

2.2. High-light treatment

The illumination of isolated PSII particles was carried out under continuous stirring at room temperature (22°C) with white light. The Chl concentration during illumination was 100 μg/ml. The membranes were illuminated on the vessel surface with intensity of 2400 μE m$^{-2}$ s$^{-1}$ and 1250 μE m$^{-2}$ s$^{-1}$ for Raman and fluorescence measurements, respectively. Samples for analysis were taken at different time periods (15 and 45 min) during illumination.

2.3. 77 K Fluorescence measurements

The 77 K chlorophyll fluorescence emission spectra were measured using a JobinYvon JY3 spectrofluorimeter. The actinic light with low enough intensity was provided by Xenon lamp “Suprasil”- 150W, with slits width of 4 nm. The experimental spectra were corrected for the spectral sensitivity of the detection system. To normalize the emission spectra of BBY 0.5 μM fluorescein (sodium salt) was added as an internal standard to the medium. At this concentration the fluorescein did not interfere with the fluorescence emission [8].

2.4. Decomposition of the fluorescence emission spectra

Detailed studies on the fluorescence of PSII complex have shown that four main bands in the 77 K PSII emission contribute to the spectrum, identified as F680, F685, F695 and F700 after their maxima positions in nm [9-13]. The first emission band, F680, is assigned to trimeric LHCII, peaking at 679 nm [10,14-16] originating from the red-most Chl $a$ pigment in LHCII. F685 arises from traps of Chl $a$ (absorbing at 683 nm) in D1-D2 RC complex and CP43, whereas F695 PSII emission from a ‘trap’ Chl $a$ (absorbing at 690 nm) in CP47 [17-19]. The fourth band, F700, is characteristic for the aggregated LHCII [10-13]. It was shown [15,16] that at 77 K the spectral shape of LHCII trimers’ fluorescence coincides with the fluorescence lineshape of monomeric Chl $a$ in a solution [20]. The emission bands F685 and F695 in PSII fluorescence also arise from monomeric Chl $a$ molecules [17-19] with different red shift in their absorption and emission maxima as compared to the maximum of trimeric LHCII, caused by various protein environment and interactions. Komura et al. [19] estimated that although the peak maximum of F685 was red-shifted, the value of its full width at half maximum (FWHM) was very close to the reported one in isolated LHCII. That is why we used the emission spectrum of trimeric LHCII for F680 and F685 spectral line shape with appropriate red shift. It was shown that the 77 K spectrum of F695 was not only red shifted as compared to the trimeric LHCII, but with FWHM about 1.5 times as broad as the trimeric LHCII one [17,19]. At critical micelle concentration (CMC) LHCsII are presented only in trimeric forms, so at this concentration the band F700 is absent and only the bands F680, F685 and F695 contribute to the total PSII spectrum. The subtraction of the sum of F680 and F685 from the total emission spectrum of PSII membranes at CMC should yield the spectral line shape of F695. To determine the scale factors and red shifts of F680 and F685 a program is made, fitting the sum of F680, F685 and F695 to the experimental PSII spectrum in the spectral range from 650 nm to 689.2 nm, where the contribution of F700 is negligibly small, using the least square method. The fitting parameters for the amplitudes of F680, F685 and F695 were the only free-running parameters. The shifts for F680 and F685 were varied in very narrow limits (0.5-1 nm) only, determined from the literature data, while the position of F695 band was fixed at the
position of the spectrum obtained at CMC concentration. Considering that the experimental PSII spectrum is composed of the four bands, we obtain the fourth band F700 as a difference between the total spectrum and the sum of F680, F685 and F695.

2.5. *Resonance Raman spectra measurements*

Room temperature Resonance Raman (RR) spectra were measured using a microRaman spectrometer (Jobin-Ivon, HR 800). The excitation was provided by an argon ion laser (Innova 307, Coherent) at 457.8, 488 and 514.5 nm. The laser intensities were 4 mW and the spectral resolution was 0.5 cm$^{-1}$ for the measurements using a grating of 1800 gr/mm.

3. Results and discussion

3.1. *Changes in 77 K fluorescence spectra*

The 77 K fluorescence emission spectra of PSII particles upon 436, 472 and 515 nm excitation are compared in figure 1 a,b,c. They are typical for PSII, having two clearly expressed maxima at 685 nm and 695 nm.

![Figure 1](image)

*Figure 1.* 77 K fluorescence spectra of control and light treated PSII particles: 1 - 0 min; 2 - 15 min and 3 - 45 min upon (a) 436, (b) 472 and (c) 515 nm excitation.

High-light treatment results in a strong quenching of the fluorescence and a red-shift of the main fluorescence maximum, from 695 nm in control particles to 695.4 nm and 699 nm after 15 and 45 min illumination with light intensity of 1250 μE m$^{-2}$ s$^{-1}$, respectively. Quenching is the strongest when the excitation is absorbed by carotenoids (figure 1c). The red shift suggests that the relative contribution of F695 and F700 to the whole spectrum is increased. In order to analyze in detail the contribution of the distinct emitting Chl $\alpha$ pools as well as their behavior during illumination, the spectra were decomposed as it was described in Materials and Methods. As an example of such decomposition, a typical fit is shown in figure 2.

The effect of the time of illumination on the components: F680, F685, F695, and F700 is presented in figure 3 a,b,c. All bands diminished during illumination indicating intensive quenching of the fluorescence and possible bleaching of Chl $\alpha$ and carotenoids.

The analysis of decomposed spectra shows that high-light treatment causes reduction of all fluorescence bands, mostly affecting the fluorescence of F685 and F695 bands. The increased relative contribution of aggregated LHCII to the whole spectrum and the progressive red shift of about 3 nm confirms that prolonged strong illumination could lead to increased energy transfer to the Chl $\alpha$ pools, emitting at 700 nm and enhanced extent of LHCII aggregation [16,21].
Figure 2. Typical decomposition of the 77 K fluorescence spectrum with four components F680, F685, F695 and F700. The sum of F680, F685 and F695 is shown with dashed line.

Figure 3. Effect of the duration of illumination: solid line - 0 min, dashed line - 15 min, dotted line - 45 min on the components: F680, F685, F695, and F700 upon (a) 436, (b) 472 and (c) 515 nm excitation.
3.2. Changes in the resonance Raman spectra

We used RR scattering in an attempt to investigate possible photobleaching of carotenoid molecules presenting RR spectra of plant BBY particles at room temperature for the first time in figure 4. The spectra contained the four main bands (called from $\nu_1$ to $\nu_4$) characteristic of carotenoids [22,23]. The main carotenoid bands have been assigned [22,23] as follows: $\nu_1$ – to C=C bonds in phase stretching vibrations; $\nu_2$ – to C14-C15 stretches coupled to C15-H in plane bending; $\nu_3$ – to methyl CH3 in plane rocking vibrations; $\nu_4$ – to C-H out of plane bending modes coupled with C7=C8 torsion.

Light treatment did not cause any change in the shape of the Raman spectra. Some decrease in the intensity was observed only for the spectra upon 514.5 nm excitation comparing to the spectra upon 488 nm excitation, for which almost no change was observed.

![Figure 4. Raman spectra of control (solid line) and 45 min light treated BBY (dotted line) upon excitation: 1- 514.5 nm, 2 - 488 nm and 3 - 457.8nm. The spectra are normalized to $\nu_1$ band intensity. Spectrum of the buffer is also shown (short dotted line).](image)

![Figure 5. $\nu_4$ and $\nu_3$ Raman regions of BBY upon excitation: 1 - 514.5 nm, 2 - 488 nm and 3 - 457.8 nm; Lut: 1' - 514.5nm and β-Car: 2' - 488 nm. The spectra are normalized to $\nu_3$ band intensity. Asterisks indicate Raman bands of pyridine.](image)

In the control spectra of BBY obtained upon the different excitations the most pronounced changes were in $\nu_1$ band position and in the structure of $\nu_2$ and $\nu_4$ regions (figure 4 and figure 5). Since at RT the contribution of distinct carotenoids to RR spectra is very complex we also present RT spectra of β-Car and Lut in pyridine (table 1). The refractive index of the solvent used is the closest to that of the protein lipid environment of the pigments. Upon 514.5 nm excitation RR spectra of the two pigments mainly differ in the $\nu_1$ band position, which is further shifted for Lut and in the $\nu_2$ region where a new band, 1173 cm$^{-1}$, exists in the spectrum of β-Car. For the same excitation in the BBY spectrum $\nu_1$ appears only 2 cm$^{-1}$ shifted from $\nu_1$ of Lut and no traces of 1173 cm$^{-1}$ can be seen. Since such small shifts of $\nu_1$ in spectra of photosynthetic complexes are often related to different dielectric properties of the environment and pigment-protein interactions we can conclude that at 514.5 nm excitation the LHCII bound Lut RR spectrum is dominant. The great difference in the $\nu_4$ region and specifies that its structure is much more distorted when it is bound to BBY. The comparison for 488 nm excitation reveals that the predominant contribution in BBY spectrum comes most probably from β-Car since the position of the most intensive Raman bands in $\nu_2$ and $\nu_3$ regions resembles the spectrum of β-Car, there is a trace of 1173 cm$^{-1}$ and because the presence of neoxanthin in these complexes is low. The spectrum of BBY upon 457.8 nm mainly differs in the position of $\nu_1$ and matches the $\nu_4$ region of the spectrum upon 488 nm excitation. Since the content of β-Car is predominant compared to other carotenoids we conclude that the signal mainly comes from β-Car.
The results show that there is no significant bleaching of β-Car molecules whereas the Lut is weakly bleached. This could be explained by the shielding effect of the antenna that surrounds the core, preserving β-Car molecules from harmful action of the high-light intensities.

### Table 1. The main Raman lines in the spectra of Lut and β-Car in pyridine.

| Exc. | Pigment | $v_1$, cm$^{-1}$ | $v_2$, cm$^{-1}$ | $v_3$, cm$^{-1}$ | $v_4$, cm$^{-1}$ |
|------|---------|-----------------|-----------------|-----------------|-----------------|
| 514.5 nm | β-Car | 1520 | 1212 | 1190 | 1173 | 1156 | 1004 | 969 | 962 | 955 |
| | Lut | 1522 | 1212 | 1190 | 1156 | 1004 | 972 | 966 | 954 |
| 488 nm | β-Car | 1524 | 1213 | 1189 | 1173 | 1156.5 | 1005.5 | 972 | 966 | 956 |
| | Lut | 1524 | 1211 | 1187 | 1153.5 | 1002 | 965 | 952 |

**Acknowledgements**

This study was supported by Grants: No. DO02-167-2008 from the National Science Fund of the Ministry of Education and Science of Bulgaria and No. 050-2010 from the Scientific Research Foundation at Sofia University, Bulgaria.

**References**

[1] Mc Evoy J P and Brudvig G W 2006 *Chem. Re.* **106** 4455
[2] Messinger J and Renger G 2007 *Primary Processes of Photosynthesis: basic principles and apparatus* vol II, ed G Renger (Cambridge: Royal Society Chemistry) pp 295-352
[3] Renger G 2007 *Photosyn. Res.* **92** 407
[4] Dekker J P and Boekema E J 2005 *Biochim. Biophys. Acta* **1706** 12
[5] Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A and Saenger W 2009 *Nature Struct. Mol. Biol.* **16** 1068
[6] Berthold D A, Babcock G T and Yocum C F 1981 *FEBS Lett.* **134** 231
[7] Lichtenthaler H K 1987 *Methods Enzymol* vol 148, eds S P Colowick, N O Kaplan, L Packer and R Donce (New York: Academic Press) pp 350–382
[8] Krause G H, Briantais J M and Vernotte C 1983 *Biochim.Biophys. Acta* **723** 169
[9] van Grondelle R, Dekker J P, Gillbro T and Sundström V 1994 *Biochim. Biophys. Acta* **1187** 1
[10] Ruban A V, Calkoen F, Kwa S L S, van Grondelle R, Horton P and Dekker J P 1997 *Biochim. Biophys. Acta* **1321** 61
[11] Andreeva A, Stoitchkova K, Busheva M and Apostolova E 2003 *J. Photochem. Photobiol. B* **70** 153
[12] Stoitchkova K, Busheva M, Apostolova E and Andreeva A 2006 *J. Photochem. Photobiol. B* **83** 11
[13] Haferkamp S, Haase W, Pascal A, van Amerongen H and Kirchhoff H 2010 *J. Biol. Chem.* **285** 17020
[14] Hemerlijr P W, Kwa L S, van Grondelle R and Dekker J P 1992 *Biochim. Biophys. Acta* **1098** 159
[15] Palacios M A, De Weerd F, Ihalainen J A, van Grondelle R and van Amerongen H 2002 *J. Phys. Chem.* **106** 5782
[16] Andreeva A, Abarova S, Stoitchkova K and Busheva M 2009 *Eur. Biophys. J.* **38** 199
[17] Andrizhiyevskaya G E, Chojnicka A, Bautista J A, Diner B A, van Grondelle R and Dekker J P 2005 *Photosynth. Res.* **84** 173
[18] Krausz E, Hughes J L, Smith P J, Pace R J and Arskold S P 2005 *Photosynth. Res.* **84** 193
[19] Komura M, Shibata Y and Itoh S 2006 *Biochim. Biophys. Acta* **1757** 1657
[20] Boardman N K and Thorne S W (1971) *Biochim. Biophys. Acta* **4618** 222
[21] Van Oort B, van Hoek A, Ruban A V and van Amerongen H 2007 *FEBS Lett.* **581** 3528
[22] Robert B 2009 *Photosynth. Res.* **101** 147
[23] Koyama Y and Fujii R 1999 *The Photochemistry of Carotenoids* eds H Frank, A Young, G Britton and R Cogdell (Dordrecht: Kluwer Acad. Publ.) pp. 161-188