JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS, Vol. 11, No. 9, September 2009, p. 1234 - 1237

Effect of structural changes in Photosystem II supercomplexes on their fluorescence properties♣

A. ANDREEVA*, K. STOITCHKOVA, S. ABAROVA, M. BUSHEVA[a]
Sofia University, Faculty of Physics, Department of Condensed Matter Physics, 5, J. Bourchier blvd., 1164 Sofia, Bulgaria
[a]Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev str. bl.21, 1113 Sofia, Bulgaria

Photosystem II is a multi-subunit pigment-protein complex embedded in the chloroplast thylakoid membranes. It consists of a large number of extrinsic and intrinsic proteins. In this report, the effect of the removal of extrinsic proteins of the oxygen-evolving complex, and several amino acids, from surface exposed polypeptides and/or small proteins on the efficiency of energy transfer and its regulation is investigated, by using chlorophyll *a* fluorescence at 77 K. The obtained results show that the two treatments have opposite effects on the photosystem II fluorescence. The removal of small surface exposed proteins enhances the total photosystem II fluorescence, influencing mainly the highly fluorescing trimeric outer antenna. The removal of extrinsic proteins of the oxygen-evolving complex results in a decrease in the total fluorescence intensity. The decrease is explained by a reduction of the distance from outer antenna to inner antenna CP43 and from outer antenna to proteins in the reaction center complex. This also diminishes the distances between chl *a* molecules and changes their mutual orientation, thus leading to possible concentration quenching of the chlorophyll fluorescence. For both treatments, the impact of the aggregation extent of outer antenna, controlled by the detergent concentration, is compared.

(Received November 5, 2008; accepted December 15, 2008)

Keywords: Photosystem II, 77 K chlorophyll fluorescence, structural changes

1. Introduction

The Photosystem II (PSII) super-complexes in green plants have the unique capacity to evolve oxygen from water. They are membrane-bound complexes, formed by the photosynthetic pigments: chlorophylls (Chl) and carotenoids interacting non-covalently with proteins and lipids. This large multi-subunit membrane protein complex consists of an inner antenna complex (PSII core), a peripheral trimeric light-harvesting complex (LHCII), some small monomeric peripheral proteins such as CP26, CP29 and others, and three extrinsic proteins of the oxygen-evolving complex (OEC) [1,2]. The three proteins of OEC are bound to the lumenal surface as a tetrameric cluster on the dimeric PSII core, containing the reaction center (RC), proteins D1 and D2 and the inner antenna proteins CP43 and CP47 [2]. The removal of extrinsic OEC by Tris washing changes the overall supra-molecular organization of PS II, reducing the distance from LHCII to CP43 by about 0.8 nm and from LHCII to D1 by about 1.3 nm, and in addition destabilizes the monomer–monomer interaction in the dimeric PSII core [3]. The most prominent feature of PSII digestion by trypsin is the reduced mass of LHCII [4,5] owing to the removal of several amino acids from surface exposed polypeptides and/or small proteins. But as has been shown, the OEC is resistant to trypsin cleavage [6].

Fluorescence at 77 K gives valuable information on the structural organization of the photosynthetic apparatus [7]. There are two main bands in 77 K PS II emission, generally identified as F685 and F695 after their maxima positions in nm [8-10]. The F695 PS II emission arises from the 'trap' Chl *a* (absorbing at 690 nm) in CP47, whereas F685 comes from traps Chl *a* (absorbing at 683 nm) in D1-D2 RC complex and CP43 [8-10]. More detailed studies of the fluorescence of PS II complex have shown that besides the bands F685 and F695, two other hidden bands, ascribed to the LHC II, contribute to the spectrum [7, 11-13]. The third emission band is that of trimeric LHCII, F680, originating from the most red Chl *a* pigment in LHC II, absorbing at 676 nm [7,11], while the fourth band F700, is characteristic of the aggregated LHCII [11-13].

In this report, the effect of the changes in the PS II structural organization induced by trypsin and the removal of OEC on the efficiency of energy transfer and regulation

♣ Paper presented at the International School on Condensed Matter Physics, Varna, Bulgaria, September 2008

of the amount of energy transferred to the RC, is investigated by using chlorophyll *a* fluorescence at 77 K. For both treatments, the impact of the aggregation extent of LHCII, controlled by the detergent concentration, is also compared. Comparing the fluorescence bands attributed to PS II and their changes, induced by both treatments, we provide a basis for better understanding of the relationships between the structural organization of the pigment-protein complexes and the steady state spectroscopic properties of the interacting pigments in the Photosystem II complexes. We also sought to elucidate the role of peripheral LHC II aggregated states.

2. Experimental

2.1. Isolation of PSII submembrane particles

The PSII enriched subchloroplast fraction was isolated from market spinach, following the modified procedure of Berthold et al. [14] used by Chapman et al. [15]. The PSII particles were re-suspended in media containing 20 mM Hepes (pH 7.5), 15 mM NaCl, 5 mM $MgCl_2$, 0.4 M sucrose and stored at -20^oC.

The aggregation state of the complexes was varied by adding desired concentrations of the used detergent *n*-dodecyl *β*-D-maltoside (DM) in samples diluted to 10 μg chl/ml. The sample was suspended in buffer media with 40 % glycerol. To normalize the emission spectra of PSII particles, 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 [16]. The chlorophyll content of the samples was estimated using the Lichtenthaler method [17].

2.2. Proteolytic and Tris treatments

PSII membranes were washed in a low salt medium and incubated with trypsin (20 mg/mg chl) for 10 min in the dark at room temperature [18]. The enzyme action was stopped by addition of trypsin inhibitor in 20-fold excess. The removal of 18, 24 and 33 kDa proteins of the oxygen evolving complex was achieved by alkaline 1 M Tris treatment [19].

Trypsinized and depleted PSII membranes were washed by centrifugation and stored in buffer media for further measurements.

2.3. 77 K Chl fluorescence measurements

The 77 K chlorophyll fluorescence emission spectra were obtained in a translucent Dewar using a double monochromator spectrometer (model 1403, Spex) as described earlier [12,13]. Excitation was provided by an argon ion laser (Inova 307) at λ = 488 nm. The experimental spectra were corrected for the spectral sensitivity of the detection system.

3. Results and discussion

3.1. Changes in the 77 K fluorescence spectra of trypsin treated PSII membranes

The low temperature fluorescence spectra of trypsin treated PSII particles, and the effect of the detergent DM addition, are compared in Fig. 1.

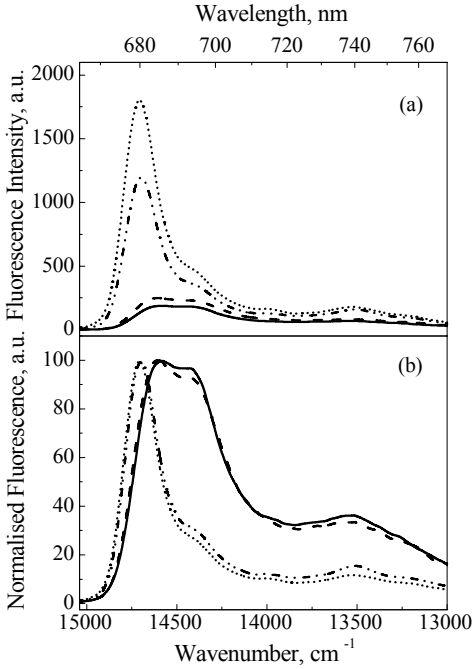

Fig. 1. 77 K fluorescence emission spectra of control (solid line) and trypsin treated (dash line) PSII particles (a) and the same spectra, normalized to their maxima (b). The effect of the detergent DM addition is also shown in the control (dash dot dot line) and trypsin treated (short dot line) PSII particles.

It is clearly seen that the trypsinization causes an increase in the total fluorescence intensity which is further enhanced after addition of DM. Similarly, the changes in the spectral shape (Fig. 1b, where the spectra were normalized to their maxima) are much more pronounced after the DM treatment of the control and trypsin treated PSII particles. The trypsinization alone has caused an apparent blue-shift of the spectrum and a relative decrease of F695, due to the removal of small surface exposed proteins [4-6]. The detergent, reducing the aggregation extent of LHCII, leads to a strong enhancement of the fluorescence, since the trimeric LHC II is highly fluorescing.

3.2. Changes in the 77K fluorescence spectra of Tris washed PSII membranes

In contrast to the trypsin action, the effect of the removal of extrinsic OEC on the PSII low temperature fluorescence is a decrease in the total fluorescence

intensity and in more pronounced changes in the shapes of the spectra (Fig. 2).

It seems that the Tris washing reduces the LHCII fluorescence and increases relatively the F695. The addition of DM also induces further changes in the fluorescence in PSII particles, but the effect is weaker in depleted PSII membranes than in the control ones.

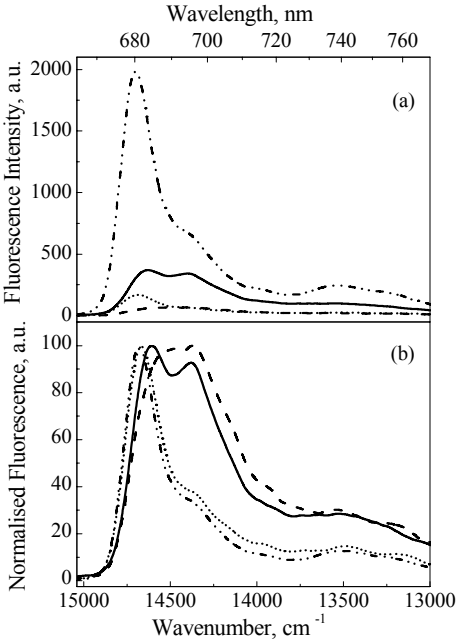

Fig. 2. 77 K fluorescence emission spectra of control (solid line) and Tris treated (dash line) PSII particles (a) and the same spectra, normalized to their maxima (b). The effect of the detergent DM addition is also shown in control (dash dot dot line) and trypsin treated (short dot line) PSII particles.

3.3. Comparison of the changes in the PS II fluorescence bands induced by both treatments

To further analyze and compare the data, we performed deconvolutions of the spectra from Figs. 1 (a) and 2 (a), based on a procedure described in [12,13]. The experimental emission spectra are fitted with the sum of the four main bands (F680, F685, F695 and F700) and one small vibrational sub-band, using the least squares method. A typical fit is shown in Fig. 3, as an example of the decomposition.

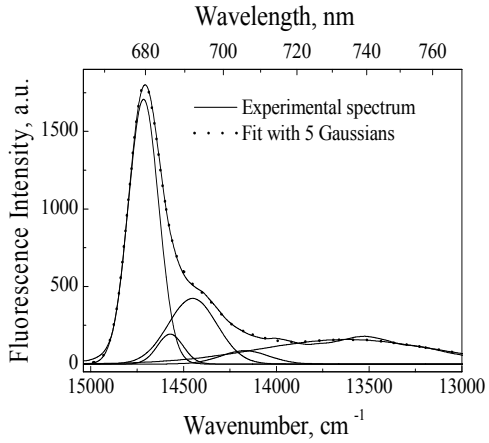

Fig. 3. A typical fit for the decomposition of the 77 K PS II fluorescence emission spectrum, with 5 Gaussians.

The ratios of each main fluorescence band of the treated to the non treated PSII membranes, estimated by the decomposition, are given in Table 1. They reveal that the trypsin-induced fluorescence increase is mostly due to the increase in the fluorescence of LHCII: F680 and F700. The enhanced effect of DM in trypsin treated BBY might be due to a decrease in the energy transfer efficiency between the LHC and the inner antennae and the RC, and between the inner antennae and the RC themselves.

Table 1. Ratios of the four main fluorescence bands of the treated to the control PSII membranes, estimated by the decomposition of the spectra

Main bands	Ratios without DM	
	Trypsin	Tris washed
F680	1.47	0.14
F685	1.22	0.19
F695	1.27	0.2
F700	1.49	0.26

The decomposition of the spectra of the Tris treated PSII membranes shows that this treatment causes a decrease of all fluorescence bands, mostly affecting the fluorescence of the monomeric and trimeric forms of LHCII (F680). Again the DM treatment seems to induce further changes in the energy transfer efficiency affecting the transfer between the aggregated LHCII and the inner antennae and between the inner antennae and the RC. These changes can be explained by the structure of the peripheral antenna after Tris washing being less stable and more vulnerable to detergent-induced dissociation.

4. Conclusions

We have examined the effect of structural changes on the 77 K fluorescence of Photosystem II particles under trypsin and Tris washing treatments. The results show that the two treatments have opposite effects on the PSII fluorescence. A possible reason for such a behaviour is

that the removal of small surface exposed proteins [4, 5], influencing mainly the highly fluorescing LHCII, enhances the total PSII fluorescence, while reduction of the distance from LHCII to CP43 and from LHCII to D1 [3] also diminishes the distances between chl *a* molecules and changes their mutual orientation, thus leading to possible concentration quenching of the chlorophyll fluorescence. For both treatments, the aggregation extent of LHCII, controlled by detergent concentration, has a strong impact on the fluorescence, enhancing predominantly the fluorescence of trimeric and monomeric LHCII.

Acknowledgements

This study was supported by Grant No.11 (Program "Support for the research activities at the universities") from the Scientific Research Fund of the Ministry of Education and Science of Bulgaria and by Grant No.16 from the Scientific Research Foundation at Sofia University, Bulgaria.

References

[1] J. P. Dekker, E. J. Boekema, Biochim. Biophys. Acta **1706**, 12 (2005).
[2] J. Nield, E. V. Orlova, E. P. Morris, B. Gowen, M. van Heel, J. Barber, Nature Struct. Biol. **7,** 44 (2000).
[3] E. J. Boekema, J. F. L. van Breemen, H. van Roon, J. P. Dekker, Biochem. **39**, 12907 (2000).
[4] D. P. Carter, L. A. Staehelin, Arch. Biochem. Biophys. **200**, 364 (1980).
[5] D. P. Carter, L. A. Staehelin, Arch. Biochem. Biophys **200**, 374 (1980).
[6] S. K. Hong, S. A. Pawlikowski, K. A. Vander Meulen, C. F. Yocum, Biochim. Biophys. Acta **1504**, 262 (2001).
[7] R. van Grondelle, J. P. Dekker, T. Gillbro, V. Sundstrom, Biochim. Biophys. Acta **1187**, 1 (1994).
[8] E. G. Andrizhiyevskaya, A. Chojnicka, J. A. Bautista, B. A. Diner, R. van Grondelle, J. P. Dekker, Photosynth. Res. **84**, 173 (2005).
[9] E. Krausz, J. L. Hughes, P. J. Smith, R. J. Pace, S. P. Årsköld, Photosynth. Res. **84**, 193 (2005).
[10] M. Komura, Y. Shibata, S. Itoh, Biochim. Biophys. Acta **1757**, 1657 (2006).
[11] A. V. Ruban, F. Calkoen, S. L. S. Kwa, R. van Grondelle, P. Horton, J. P. Dekker, Biochim. Biophys. Acta **1321**, 61 (1997).
[12] A. Andreeva, K. Stoitchkova, M. Busheva, E. Apostolova, J. Photochem. Photobiol. B **70**, 153 (2003).
[13] K. Stoitchkova M. Busheva, E. Apostolova, A. Andreeva, J. Photochem. Photobiol. B **83**, 11 (2006).
[14] D. A. Berthold, G. T. Babcock, C. F. Yocum, FEBS Lett. **134**, 231 (1981).
[15] D. J. Chapman, K. Gonaris, J. Barber, Biochim. Biophys. Acta **933**, 423 (1988).
[16] G. H. Krause, J. M. Briantais, C. Vernotte, Biochim. Biophys. Acta **723**, 169 (1983).
[17] H. K. Lichtenthaler, Methods Enzymol. **148**, 350 (1987).
[18] S. G. Taneva, M. C. Busheva, A. G. Dobrikova, I. B. Petkanchin, J. Photochem. Photobiol. B **23**, 19 (1994).
[19] T. Kuwabara, N. Murata, Plant Cell Physiol. **24**, 1 (1983).

*Corresponding author: andreeva@phys.uni-sofia.bg