Short Communication

Purification of C-phycocyanin from Spirulina (Arthrospira) fusiformis

K.M. Minkova a,*, A.A. Tchernov b, M.I. Tchorbadjieva c, S.T. Fournadjieva a, R.E. Antova c, M.Ch. Busheva d

a Institute of Plant Physiology, Acad. M. Popov, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
b National Centre of Infectious and Parasitic Diseases, Y. Sakazov Blvd 26, Sofia 1504, Bulgaria
c Faculty of Biology, Sofia University, Dr. Zankov Str. 8, Sofia 1421, Bulgaria
d Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria

Received 21 June 2002; received in revised form 12 December 2002; accepted 19 December 2002

Abstract

C-phycocyanin was purified from Spirulina (Arthrospira) fusiformis by a multi-step treatment of the crude extract with rivanol in a ratio 10:1 (v/v), followed by 40% saturation with ammonium sulfate. After removal of rivanol by gel-filtration on Sephadex G-25, the pigment solution was saturated to 70% with ammonium sulfate. After the last step of purification, C-phycocyanin had an emission and absorption maxima at 620 and 650 nm, respectively and absorbance ratio $A_{620}/A_{600}$ of 4.3, which are specific for the pure hileprotein. Its homogeneity was demonstrated by sodium dodecyl sulfate-polycrylamide gel electrophoresis, yielding two bands of molecular masses 19,900 and 21,500 kDa, corresponding to α and β subunits of the pigment, respectively. The yield of C-phycocyanin was 46% from its content in the crude extract.

Keywords: Spirulina (Arthrospira) fusiformis; C-phycocyanin; Rivanol; Purification

Structural Rearrangements in Chloroplast Thyylkoid Membranes Revealed by Differential Scanning Calorimetry and Circular Dichroism Spectroscopy: Thermal-optic Effect

Anelia G. Dobrikova 1, Zauszanna Várnokyi 1, Sasha B. Krumova 2,3, László Kovács 3, Georgi K. Kostov 1, Svetla J. Todanova 1, Miral C. Busheva 1, Stefka G. Tanteva 1, and Gyöző Garab 4,8

Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Bl. 21, Sofia 1113, Bulgaria, and Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary

Received May 27, 2003; Received Manuscript Received August 5, 2003

ABSTRACT: The thermo-optic mechanism in thylkoid membranes was earlier identified by measuring the thermal and light stabilities of pigment arrays with different levels of structural complexity [Cach, E., et al. (2000) Biochemistry 39, 15250–15257]. (According to the thermo-optic mechanism, fast local thermal transients, arising from the dissipation of excess, photosynthetically not used, excitation energy, induce elementary structural changes due to the “built-in” thermal instabilities of the given structural units.) The same mechanism was found to be responsible for the light-induced trimer-to-monomer transition in LHClII, the main chlorophyll a/b light-harvesting antenna of photosystem II (PSII) [Garab, G., et al. (2002) Biochemistry 41, 15121–15129]. In this paper, differential scanning calorimetry (DSC) and circular dichroism (CD) spectroscopy on thylkoid membranes of barley and pea are used to correlate the thermo-optically inducible structural changes with well-discriminable calorimetric transitions. The thylkoid membranes exhibited six major DSC bands, with maxima between about 43 and 87 °C. The heat sorption curves were analyzed both by mathematical deconvolution of the overall endotherm and by a successive annealing procedure; these yielded similar thermodynamic parameters, transition temperature and calorimetric enthalpy. A systematic comparison of the DSC and CD data on samples with different levels of complexity revealed that the heat-induced disassembly of chiral macrodomains contributes profoundly to the first endothermic event, a weak and broad DSC band between 43 and 48 °C. Similarly to the main macrodomain-associated CD signals, this low enthalpy band could be diminished by prolonged photoinhibitory preillumination, the extent of which depended on the temperature of preillumination. By means of non-denaturing, “green” gel electrophoresis and CD fingerprinting, it is shown that the second main endotherm, around 60 °C, originates to a large extent from the monomerization of LHClII trimers. The main DSC band, around 70 °C, which exhibits the highest enthalpy change, and another band around 75–77 °C relate to the dismantling of LHClII and other pigment–protein complexes, which under physiologically relevant conditions cannot be induced by light. The currently available data suggest the following sequence of events of thermo-optically inducible changes: (i) unstacking of membranes, followed by (ii) lateral disassembly of the chiral macrodomains and (iii) monomerization of LHClII trimers. We propose that thermo-optical structural reorganizations provide a structural flexibility, which is proportional to the intensity of the excess excitation, while for their localized nature, the structural stability of the system can be retained.
Changes in the energy distribution between chlorophyll–protein complexes of thylakoid membranes from pea mutants with modified pigment content

I. Changes due to the modified pigment content

Atanaska Andreeva, Katerina Stoitchkova, Mira Busheva, Emilia Apostolova

Department of Condensed Matter Physics, Faculty of Physics, Sofia University, 5 J. Bouchier Blvd., 1164 Sofia, Bulgaria
Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. bl. 21, 1113 Sofia, Bulgaria

Received 15 July 2002; received in revised form 2 April 2003; accepted 15 May 2003

Abstract

The low-temperature (77 K) emission and excitation chlorophyll fluorescence spectra in thylakoid membranes isolated from pea mutants were investigated. The mutants have modified pigment content, structural organization, different surface electric properties and functions [Dobrilova et al., Photosynth. Res. 65 (2000) 165]. The emission spectra of thylakoid membranes were decomposed into bands belonging to the main pigment protein complexes. By an integration of the areas under them, the changes in the energy distribution between the two photosystems as well as within each one of them were estimated. It was shown that the excitation energy flow to the light harvesting, core antenna and RC complexes of photosystem II increases with the total amount of pigments in the mutants, relative to the that to photosystem I complexes. A reduction of the fluorescence ratio between aggregated trimers of LHC II and its trimeric and monomeric forms with the increase of the pigment content (chlorophyll a, chlorophyll b, and lutein) was observed. This implies that the closer packing in the complexes with a higher extent of aggregation regulates the energy distribution to the PS II core antenna and reaction centers complexes. Based on the reduced energy flow to PS II, i.e., the relative increased energy flow to PS I, we hypothesize that aggregation of LHC II switches the energy flow toward LHC I. These results suggest an additive regulatory mechanism, which redistributes the excitation energy between the two photosystems and operates at non-excess light intensities but at reduced pigment content.

© 2003 Elsevier B.V. All rights reserved.

Keywords: 77 K chlorophyll fluorescence; Energy distribution; Thylakoid membranes; Pigment–protein complexes; Pea mutants
Resonance Raman Spectroscopy of Xanthophylls in Pigment Mutant Thylakoid Membranes of Pea

Atanaska Andreeva, Katerian Stoitchkova, Mira Busheva, Emilia Apostolova, Zsuzsanna Varkonyi, Gyözö Garab

1 Sofia University, Faculty of Physics, Department of Condensed Matter Physics, 5, J. Bourcher Blvd., 1164 Sofia, Bulgaria
2 Institute of Biophysics, Bulgarian Academy of Sciences, Acad. Z. Bonchev str. bl.21, 1113 Sofia, Bulgaria
3 Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, P. O.Box 521, H-6701 Hungary

Abstract: Low-temperature resonance Raman spectroscopy was used to study the changes in the molecular structure and configuration of the major xanthophylls in thylakoid membranes isolated from mutant and pea with modified pigment contents and altered structural organization of their pigment-protein complexes. The Raman spectra, gentisitiely for known groups of bands, νC=O, which could be assigned to originate mainly from the long wavelength absorbing bands of neurosporin apoprotein 514.5 nm and 488 nm excitations, respectively. The overall configuration of these bound xanthophyll molecules in the mutants appeared to be similar to the wild type and the configuration in the wild type was almost identical with that in the isolated main chlorophyll a/b light harvesting protein complex of photosystem II (LHCl). Significant differences were found mainly in the region of νC=O (around 960 cm⁻¹), which suggest that the macroorganization of PS II-LHCl supercomplexes and of the LHCl-only domains are modified in the mutants compared to the wild type. © 2004 Wiley Periodicals, Inc. Biopolymers 0: 000–000, 2004

Keywords: resonance Raman spectroscopy; xanthophylls; thylakoid membranes; pigment mutants; light harvesting complex photosystem II

Kinetic nature of the thermal destabilization of LHClII macroaggregates

Sassika B. Krumova, Svetla J. Todinova, Mira C. Busheva, Stefka G. Taneva

Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 24, 1125 Sofia, Bulgaria

Received 28 September 2004; revised in revised form 8 November 2004; accepted 19 November 2004
Available online 1 January 2005

Abstract: The main light-harvesting chlorophyll a/b pigment-protein complex of photosystem II (LHClII) in isolated state forms macroaggregates with different ultracentrifuge and lipid content. [S. N. Smolichy, V. Barada, L. Mustardy, G. Garab, Anal. Biochem. 250 (1997) 169-175]. The thermodynamic stability of highly delipitated tightly bound LHClII macroaggregates is studied by differential scanning calorimetry and fluorescence spectroscopy. The calorimetric profile of LHClII is asymmetric, the denaturation transition is taking place at around 72 °C. A shoulder, which overlaps with the main denaturation transition, appears around 58 °C. The denaturation temperature strongly depends on the scanning rate, indicating the kinetic nature of the thermal destabilization of LHClII macroaggregates. The fluorescence data prove that the thermal denaturation of LHClII is an irreversible and kinetically controlled process. © 2004 Elsevier B.V. All rights reserved.

Keywords: Light-harvesting chlorophyll a/b pigment-protein complex; Differential scanning calorimetry; Denaturation transition; Calorimetric analysis; Fluorescence emission
BRIEF COMMUNICATION

Influence of short-term osmotic stress on the photosynthetic activity of barley seedlings

K.V. KOCHEVA*, I. M. BUSHEVA**, G. I. GEORGIEV*, P. H. LAMBREV** and V. N. GOLTSEV***

Institute of Plant Physiology* and Institute of Biophysics**, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 21, BG-1113 Sofia, Bulgaria
Biological Faculty, University of Sofia, 8 D. Tsankov Blvd., BG-1164 Sofia, Bulgaria***

Abstract

Oxygen evolution and chlorophyll a fluorescence transients of two barley (Hordeum vulgare L.) cultivars subjected to polyethylene glycol induced osmotic stress was examined. The relative water content of the plants was used as a measure of their water status. The results suggested that although dehydration was considerable, photosystem 2 was weakly affected by the osmotic treatment.

Additional key words: chlorophyll fluorescence, Hordeum vulgare, oxygen evolution, polyethylene glycol 8000, relative water content.

Changes in the energy distribution in mutant thylakoid membranes of pea with modified pigment content. II. Changes due to magnesium ions concentration

Katerina Stoitchkova a, Mira Busheva b, Emilia Apostolova b, Atanaska Andreeva a, b

a Sofia University, Faculty of Physics, Department of Condensed Matter Physics, S. J. Bouchev Blvd., 1164 Sofia, Bulgaria
b Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev str. Bl. 21, 1113 Sofia, Bulgaria

Received 25 July 2005; received in revised form 22 November 2005; accepted 23 November 2005
Available online 10 January 2006

Abstract

Low-temperature (77 K) steady-state chlorophyll fluorescence emission spectra, room temperature fluorescence and light scattering of thylakoid membranes isolated from pea mutants were studied as a function of Mg" concentration. The mutants have modified pigment content and altered structural organization of the pigment-protein complexes, distinct surface electric properties and functions. The analysis of the 77 K emission spectra revealed that Mg" depletion of the medium caused not only an increased energy flow toward photosystem I in all investigated membranes but also changes in the quenching of the fluorescence, most probably by internal conversion. The results indicated that the macroorganization of the photosynthetic apparatus of mutants at supramolecular level (distribution and segregation of two photosystems in thylakoid membranes) and at supramolecular level (stacking of photosystem II supercomplexes) required different Mg ion concentrations. The data confirmed that the segregation of photosystems and the stacking of thylakoid membranes are two distinct phenomena and elucidated some features of their mechanisms. The segregation is initiated by changes in the lateral microorganization of light harvesting complexes II, their migration (repulsion from photosystem I) and subsequent separation of the two photosystems. Most likely 3D aggregation and formation of macromodomains, containing only photosystem II antenna complexes, play a certain precuratory role for the increasing degree of the membrane stacking and the energy coupling between the light harvesting complexes II and the core complexes of photosystem II in the frame of photosystem II supercomplexes.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Energy distribution; Thylakoid membranes; Pigment-protein complexes; Pea mutants; Mg ions; Stacking; Separation of PS I and PS II
Improved procedure for separation and purification of Arthronema africanum phycobiliproteins

Kaledona Minkova · Magdalena Tchorbadjieva · Alcey Tchernov · Margarita Stoianova · Liliana Gigova · Mira Busheva

Received: 22 September 2006 / Revised: 23 November 2006 / Accepted: 27 November 2006 / Published online: 6 January 2007
© Springer Science+Business Media B.V. 2007

Abstract A rapid, inexpensive and reliable procedure for separation and purification of C-phycocyanin (C-PC) and allophycocyanin (APC) from Arthronema africanum based on a previously described rivanol-sulfate method for C-PC purification was developed. Exclusion of NaCl from the extraction buffer resulted in complete separation of APC and C-PC, two-fold reduction of rivanol treatments, and a higher yield and purity of C-PC. Pure C-PC (A420/A260 of 4.52) and APC (A655/A260 of 2.41) were obtained. The estimated molecular masses of the α and β subunits were 17 and 19 kDa for C-phycocyanin and 16 and 18 kDa for APC, respectively. The overall C-PC recovery of 55% (w/w) from its content (100 mg) in the crude extract was 10–20% higher than so far reported. The procedure appears promising for scaling up and broader applications.

Keywords Allophycocyanin · Arthronema · C-PC · Extraction · Phycobiliproteins · Separation

Introduction Phycobiliproteins (PBPs) are photosynthetic pigments of cyanobacteria, red algae and cryptomonads. They are divided into three classes:

Laser fluorescence spectroscopy of the light harvesting complex II in different aggregation states

Atanaska Andreeva a, Bilyana Vapirova a, Mira Busheva b
bInstitute of Biophysics, Bulg. Acad. Sci., Acad. G. Bonchev Str., bl. 21, 1113 Sofia, Bulgaria

ABSTRACT

In order to elucidate the molecular mechanism of the non-photochemical quenching of the excess light energy – quenching of excited chlorophyll a singlet states, low-temperature (77 K) steady-state laser fluorescence emission spectroscopy was applied to the main chlorophyll a/b protein light harvesting complex of photosystem II in different aggregation stages. The aggregation of the complexes led to the quenching of the chlorophyll a fluorescence, as in the process of non-photochemical quenching. The quenching is concomitant with a strong broadening of the emission spectra and an appearance of a new emission band red shifted compared to the emission spectrum of the trimeric forms of the light harvesting complex of photosystem II. The aggregation state of the complexes was varied by changing the concentrations of the used detergent α-dodecyl β-D-maltoside. The low-temperature chlorophyll a emission spectra of the light harvesting complex of photosystem II were excited by laser line at 488 nm of an argon laser. Spectra were decomposed into the bands attributed to the trimeric and aggregated forms of the light harvesting complex of photosystem II. Based on the analysis of the obtained data and the new structure of light harvesting complex of photosystem II, a model describing quantitatively the quenching of the chlorophyll a fluorescence in the light harvesting complex of photosystem II in different aggregation states is proposed. The model revealed that upon aggregation besides the changes in the relative absorption of small and large aggregates, the amount of quenchers, most probably chl a dimers, and the rate constant for energy transfer to them are also changed.

Keywords: Light harvesting complex of photosystem II, fluorescence quenching, laser fluorescence spectroscopy
Quenching of the Chlorophyll a Fluorescence in the Aggregates of Light Harvesting Complex II

A. Andreeva¹, S. Abarova¹, M. Busheva²
¹University of Sofia, Faculty of Physics, 5, J. Bourchier Blvd., 1164 Sofia, Bulgaria
²Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. bl. 21, 1113 Sofia, Bulgaria

Abstract. Aggregates of the main chlorophyll a/b protein light harvesting complex of photosystem II (LHC II) were characterized by low-temperature (77 K) steady-state fluorescence spectroscopy and dynamic light scattering Malvern system-3600. The determination of the size of the aggregated samples by the Malvern system showed that the aggregated samples contain a mixture of large and small aggregates, the size of small aggregates being similar to that of trimeric forms. The low-temperature chlorophyll a emission spectra of the LHC II in different aggregation states were excited at 436 nm where mostly chlorophyll a molecules absorb. Spectra were decomposed into bands attributed to the trimeric and aggregated forms of the LHC II. The individual spectrum of large aggregates was obtained by subtraction of scaled spectrum of trimeric LHC II from the total spectrum of aggregated complexes. The results revealed that upon aggregation the quenching of the chlorophyll a fluorescence is due to the formation of the complexes containing quenchers, poorly fluorescing chlorophyll a associates emitting at 695 nm, to their growth and to the extended energy transfer to them.

PACS number: 87.84.-t
Model for fluorescence quenching in light harvesting complex II in different aggregation states

Atanaska Andreeva · Silvia Abarova · Katerina Stoitchkova · Mira Busheva

Received: 8 May 2008 / Revised: 9 September 2008 / Accepted: 10 September 2008 / Published online: 26 September 2008
© European Biophysical Societies’ Association 2008

Abstract Low-temperature (77 K) steady-state fluorescence emission spectroscopy and dynamic light scattering were applied to the main chlorophyll \(ab\) protein light harvesting complex of photosystem II (LHC II) in different aggregation states to elucidate the mechanism of fluorescence quenching within LHC II oligomers. Evidences presented that LHC II oligomers are heterogeneous and consist of large and small particles with different fluorescence yield. At intermediate detergent concentrations the mean size of the small particles is similar to that of trimers, while the size of large particles is comparable to that of aggregated trimers without added detergent. It is suggested that in small particles and trimers the emitter is monomeric chlorophyll, whereas in large aggregates there is also another emitter, which is a poorly fluorescing chlorophyll associate. A model, describing populations of antenna chlorophyll molecules in small and large aggregates in their ground and first singlet excited states, is considered. The model enables us to obtain the ratio of the singlet excited-state lifetimes in small and large particles, the relative amount of chlorophyll molecules in large particles, and the amount of quenchers as a function of the degree of aggregation. These dependencies reveal that the quenching of the chl \(a\) fluorescence upon aggregation is due to the formation of large aggregates and the increasing of the amount of chlorophyll molecules forming these aggregates.

As a consequence, the amount of quenchers, located in large aggregates, is increased, and their singlet excited-state lifetimes steeply decrease.

Keywords Light harvesting complex II · 77 K chlorophyll fluorescence · Aggregation · Fluorescence quenching

Abbreviations LHC Main chlorophyll \(ab\) protein light harvesting II complex of photosystem II chl Chlorophyll NPQ Non-photochemical quenching DM \(n\)-Dodecyl \(\beta\)-n-maltoside CMC Critical micelle concentration F680 Fluorescence band at 680 nm F700 Fluorescence band at 698 nm

Introduction

The main light-harvesting antenna complex of photosystem II (LHC II) is a pigment–lipid–protein structure where light harvesting and light dissipation are closely related processes (Jansson 2005). Dissipation of the excess light energy as heat is accomplished by quenching of excited chlorophyll (chl) \(a\) singlet states, a mechanism referred
Relationship between the degree of carotenoid depletion and function of the photosynthetic apparatus

Kolyo Dankov a, Mira Busheva a, Detelina Stefanov b, Emilia L Apostolova a, a

1 Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
2 Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria

**Abstract**

Further, an inhibitor of the carotenoid biosynthesis, was used to study the relationship between the degree of carotenoid depletion and the function of the photosynthetic apparatus. The data reveal that, at a small reduction of the carotenoid content (20% decrease of the total carotenoids), the PSII and PSI (oxidation of P700 by far-red light) photosynthesis is not influenced, while the oxygen evolution is strongly inhibited. Further reduction of the total carotenoids content (more than 40%) leads to decrease of the chlorophyll content and inhibition of the functions of both photosystems as the effect on the photosynthetic oxygen evolution and primary photosynthesis is greater than the effect on P700 oxidation. The analysis of the oxygen production under continuous illumination and flash oxygen yields suggests that the inhibition of the oxygen evolution is caused mainly by the damage of PSI centers.

© 2009 Elsevier B.V. All rights reserved.

---

BVO emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO2 and high temperature

Violeta Velikova a, b, Tsonko Tsonev a, b, Georgie Barta b, Mauro Centritto c, Dimitrina Koleva a, b, Miroska Stefanova a, b, Mira Busheva a, Francesco Loreto a

a Bulgarian Academy of Sciences, Institute of Biophysics, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
b Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
c Department of Environmental and Plant Sciences, University of Bologna, Via Massimo d’Azeglio, 80, 40126 Bologna, Italy

**Abstract**

To investigate the interactive effects of increasing [CO2] and heat stress on ozone (O3) and methanol (MeOH) emissions, *Platanus orientalis* was grown for one month in ambient (380 ppm CO2) or elevated (1000 ppm CO2) CO2 and exposed to high temperature (HT) (38°C) in its pre-existing leaves. I.E. emissions were always higher, but MeOH emissions lower as compared to newly-emerged leaves. They were both stimulated by HT. Elevated CO2 significantly reduced I.E. in both leaf types, whereas it increased MeOH in newly-emerged leaves only. In newly-emerged leaves, increased CO2 decreased photosynthesis and altered the chloroplast ultrastructure and membrane integrity. These harmful effects were amplified by HT. HT did not cause any unfavorable effects in pre-existing leaves, which were characterized by inherently higher I.E. rates. We conclude that: (1) these results further prove the ozone's putative photo-protective role of membrane; (2) HT may likely outweigh the inhibitory effects of elevated [CO2] on I.E. in the future.

© 2009 Elsevier Ltd. All rights reserved.
Effect of structural changes in Photosystem II supercomplexes on their fluorescence properties*

A. ANDREEVA*, K. STOITCHKOVA, S. ABAROVA, M. BUSHEVA*
Sofia University, Faculty of Physics, Department of Condensed Matter Physics, 5, J. Bourchier blvd., 1164 Sofia, Bulgaria
*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 chlorophyll 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

ASSESSMENT OF SENSITIVITY OF PHOTOSYNTHETIC OXYGEN EVOLUTION AND CHLOROPHYLL FLUORESCENCE PARAMETERS TO COPPER FOR APPLICATION IN BIOSENSORS

Anelia Dobrikova, Radka Vlakova, Georgy Rashkov, Mira Busheva, Amarendra N. Misra*, Emilia Apostolova

(Submitted by Academician K. Kumanov on March 12, 2009)

Abstract

The effects of copper ions on the parameters of the photosynthetic oxygen evolution measured by polarographic oxygen rate electrode and Pulse-Amplitude-Modulation (PAM) chlorophyll fluorescence of pea thylakoid membranes are compared. Data reveal that the non-photochemical quenching parameters and flash-induced oxygen evolution are suitable for detection of copper in solutions.

Key words: thylakoid membranes, photosystem II, copper, oxygen evolution, PAM fluorometry
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 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 LHCII. The analysis of RR spectra revealed the predominant contribution of β-carotene (β-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 β-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.
Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques

Violeta Velikova, Zsuzsanna Várkonyi, Milán Szabó, Liliana Maslenkova, Isabel Nogues, László Kovács, Violeta Peeva, Mira Busheva, Győző Garab, Thomas D. Sharkey, Francesco Loreto*

Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria (V.V., L.M., V.P.); Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, 6726 Szeged, Hungary (Z.V., M.S., L.K., G.G.); Institute of Agroenvironmental and Forest Biology (IBAF), National Research Council (CNR), 00015 Monterotondo, Rome, Italy (I.N.); Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria (M.B.); Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824, USA (T.D.S.); and Institute for Plant Protection (IPP), National Research Council (CNR), 50019 Sesto Fiorentino, Florence, Italy (F.L.)

Abstract

Three biophysical approaches were used to get insight into increased thermostability of thylakoid membranes in isoprene-emitting plants. Arabidopsis plants genetically modified to make isoprene and Platanus orientalis leaves, in which isoprene emission was chemically inhibited, were used. First, in the circular dichroism spectrum the transition temperature of the main band at 694 nm was higher in the presence of isoprene, indicating that the heat stability of chiral macromdomains of chloroplast membranes, and specifically the stability of ordered arrays of LHCI-PSII in the stacked region of the thylakoid grana, was improved in the presence of isoprene. Second, the decay of electrochromic absorbance changes resulting from the electric field component of the proton motive force (ΔA_{313}) was evaluated following single-turnover saturating flashes. The decay of ΔA_{313} was faster in the absence of isoprene when leaves of Arabidopsis and Platanus were exposed to high temperature, indicating that isoprene protects the thylakoid membranes against leakiness at elevated temperature. Finally, thermoluminescence measurements revealed that S2Q5 charge recombination was shifted to higher temperature in Arabidopsis and Platanus plants in the presence of isoprene indicating higher activation energy for S2Q5 redox pair, which enables isoprene-emitting plants to perform efficient primary photochemistry of PSII even at higher temperatures. The data provide biophysical evidence that isoprene improves the integrity and functionality of the thylakoid membranes at high temperature. These results contribute to our understanding of isoprene mechanism of action in plant protection against environmental stresses.

Keywords: circular dichroism, electrochromic shift, isoprene, membrane stability, thermoluminescence, thermotolerance.
EFFECTS OF ENHANCED BRASSINOSTEROID PERCEPTION ON PHOTOSYNTHESIS IN ARABIDOPSIS THALIANA LINE BRIOE


(Submitted by Corresponding Member A. Kossev on March 11, 2011)

Abstract

The relation between brassinosteroid signalling and photosynthesis in Arabidopsis thaliana is studied by comparing the photosynthetic performance of wild-type plants and BRIOE line with increased level of the brassinosteroid receptor BR1. The data reveal that enhanced brassinosteroid perception does not influence the net photosynthetic rate but leads to lower stomatal conductance and transpiration rate. Furthermore, the results presented demonstrate that BRIOE plants are characterized by lower oxygen evolution yield and alterations of the energy coupling of photosystem II core complex. While the photochemistry of photosystem II in BRIOE is not modified, the photochemical efficiency of photosystem I is reduced.

Key words: brassinosteroid perception, Arabidopsis thaliana, BRIOE, photosynthesis

EFFECT OF PH ON THE AGGREGATION OF THE MAJOR LIGHT HARVESTING COMPLEX OF PHOTOSYSTEM II

Mira Busheva¹, Sashka Krumova¹, Svetozar Stoichev¹, Iren Tzonova¹, Ilian Karadjov², Katerina Stoitchkova² and Atanaska Andreeva²

¹Institute of Biophysics and Biomedical Engineering, BAS, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria,
²Sofia University, Faculty of Physics, Department of Condensed Matter Physics, J. Bourchier blvd. 5, Sofia 1164, Bulgaria,
e-mail: andreeva@phys.uni-sofia.bg

Abstract

The main light–harvesting antenna complex of photosystem II (LHCII) is a pigment–lipid–protein structure playing major role in two interrelated processes, light harvesting and light dissipation. Dissipation of the excess light energy is accomplished by quenching of the excited chlorophyll a singlet states. Its precise molecular mechanism still remains controversial, however it is known that the aggregation state of LHCII is of crucial importance for this process.

LHCII organization is strongly dependent on the lipid content. Thus, in the present study low-temperature (77K) steady-state fluorescence emission and resonance Raman spectroscopy are applied to two types of LHCII with different lipid content: type II, resembling the LHCII organization in native photosynthetic membranes and type IV – delipidated lamellar LHCII aggregates. The aim is to investigate the structural changes induced upon protonation of the complex and to elucidate the role of the aggregation in the mechanism of fluorescence quenching within LHCII oligomers.