Acta Physiol Plant (2013) 35:975–978
DOI 10.1007/s11738-012-1122-8

SHORT COMMUNICATION

# Effect of high-light on photosynthetic apparatus with different content of anionic lipids and organization of light-harvesting complex of photosystem II

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Received: 10 February 2012 / Revised: 24 August 2012 / Accepted: 5 October 2012 / Published online: 16 October 2012


**Abstract** The effect of the high-light at 4 °C on isolated thylakoid membranes with different organization of light-harvesting complex of photosystem II (LHCII) and lipid composition was investigated. Data revealed that the decreased amount of anionic lipids, which is correlated with the increase in the oligomeric form of LHCII stabilized the photosystem II complex against low temperature photoinhibition.

**Keywords** Anionic lipids · High-light · Photoinhibition · Light-harvesting complex · Photosystem II

## Introduction

Prolonged illumination of plants with high-light causes decline of photosynthetic activity, and this phenomenon is known as photoinhibition that primarily affects the photosystem II (PSII) complex (Powles 1984; Aro et al. 1993; Vass 2011). PSII is a multiprotein complex in thylakoid membranes that catalyzes the photo-oxidation of water. PSII is surrounded by the light-harvesting complex II (LHCII) (Boekema et al. 2000). This LHCII complex is a highly adaptable structure and changes its morphology in response to light wavelength and intensity (Nicholson et al. 1996). In the short-term acclimation of the plants, the lateral migration of main LHCII along the thylakoid membranes is thought to be essential (Calberg et al. 1992), while in the long-term acclimation, LHCII content and organization in the photosynthetic membranes are often changed (Anderson 1999). Despite many studies, the role of light-harvesting antenna size of PSII in the susceptibility of photosynthetic apparatus to high-light treatment is not fully understood. Some investigators suggested that photoinhibition depends on the antenna size of PSII (Stroch et al. 2004; Kim et al. 2009), while others (Tyystjärvi et al. 1994) claimed that it does not depend on the LHCII size. These differences could be a result of different organization of the photosynthetic apparatus and conditions of the treatments. Recently it has been shown that the effects of UV-A radiation and high-light treatment in vivo depend on the degree of LHCII oligomerization (Ivanova et al. 2008; Dankov et al. 2009, 2011). On the other hand, LHCII oligomerization correlates with the amount of two anionic lipids, sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG) (Dankov et al. 2009, 2011). These anionic lipids are essential for the function and stability of PSII (Domonkos et al. 2008; Sato et al. 2003). In the previous investigations, it was shown that the degree of unsaturation of the membrane lipids (Gombos et al. 1994; Kanervo et al. 1995; Moon et al. 1995; Tasaka et al. 1996) affects the rate of photoinhibition, which is also influenced by light intensity, time of treatment and the temperature during high-light treatment (Aro et al. 1990, 1993). The increased sensitivity to light at low temperature is caused by the reduction in the rate of recovery (Gombos et al. 1994; Kanervo et al., 1995). At the same time, it has been shown that low temperature protects intact isolated thylakoids of higher plants against photoinhibition (Tyystjärvi et al. 1994).

The aim of the present investigation is to study the influence of light stress in vitro on the thylakoid membranes

Communicated by Z. Gombos.

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with different size and organization of LHCII as well as with different lipid composition. Two wild types and three mutants with different ratio of oligomeric to monomeric forms of LHCII (LHCIIo/LHCIIm) and amount of anionic lipids (L, in mol % from total lipids) were used: mutant *Chlorotica* XV/1422 (LHCIIo/LHCIIm = 2.45; $L = 19$), Auralia wild type (LHCIIo/LHCIIm = 2.82; $L = 17$), mutant *Costata* 2/133 (LHCIIo/LHCIIm = 3.34; $L = 16$), Borec wild type (LHCIIo/LHCIIm = 4.57; $L = 14$) and *Coreuleovireus* 2/16 (LHCIIo/LHCIIm = 6.62) (Dobrikova et al. 2000, 2001; Apostolova et al. 2006; Dankov et al. 2009, 2011). Earlier studies of the above plants also revealed that the amount of LHCII proteins in Auralia wt and its mutant *Chlorotica* XV/1422 is smaller in comparison with Borec wt and its mutants (*Costata* 2/133 and *Coreuleovireus* 2/16) (Apostolova et al. 2006). The data in the present investigation reveal that the oligomerization of LHCII, which correlates with the amount of anionic lipids, influences the degree of damage of isolated thylakoid membranes during low temperature photoinhibition.

## Materials and methods

### Preparation of thylakoid membranes

Thylakoid membranes were isolated from *Pisum sativum* L. cv. Borec and Auralia and their mutants (*Coreuleovireus* 2/16, *Costata* 2/133 and *Chlorotica* XV/1422). The plants were grown under controlled conditions with 16 h light/8 h dark photoperiod. The mutants used in this study are well defined and stable. Previously, protein, lipid and pigment composition as well as the functions, and physicochemical properties of the above mutants were characterized (Dobrikova et al. 2000, 2001; Apostolova et al. 2006; Dankov et al. 2009, 2011). The thylakoid membranes were isolated as described in (Steinback et al. 1979) and suspended in a medium containing 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$, 400 mM sucrose and stored in the refrigerator at 4 °C before measurement. The total chlorophyll concentration was determined by the method of Lichtenthaler (1987).

### High-light treatment of isolated thylakoid membranes

Thylakoid membranes were suspended in a buffer containing: 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose at chlorophyll concentration 500 μg $ml^{-1}$, forming about 1 mm thin suspension layer at continuous stirring. Thylakoid membranes were illuminated for 0–120 min in a Petri dish at 4 °C with white light (1,300 μmol $m^{-2}$ $s^{-1}$). The control samples (non-illuminated) are kept in dim light at 4 °C.

### Low temperature (77 K) chlorophyll fluorescence

Low temperature (77 K) chlorophyll fluorescence measurements were performed in a cylindrical quartz cuvette in a medium containing 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose. The chlorophyll concentration was 10 μg $ml^{-1}$. The samples were quickly frozen by plunging them in liquid nitrogen. Fluorescence spectra were recorded from 600 nm to 780 nm using Jobin–Yvon JY3 spectrofluorimeter equipped with a red-sensitive photomultiplier (Hamamatsu R928) and a liquid nitrogen device. The width of the exciting and measuring slits was 4 nm. The data were digitized by an in-built A/D converter and transferred to IBM-compatible computer for further analysis. The chlorophyll fluorescence was excited either at 436 nm (Chl *a*) or at 472 nm (Chl *b*).

### Photosynthetic oxygen evolution

Steady-state oxygen evolution (photochemical activity of PSII) was measured polarographically with a Clark-type electrode (Model DW1, Hansatech, Instruments Ltd. King's Lynn, Norfolk) in temperature-controlled cuvette at 20 °C, using saturated white light. The PSII activity was measured by the rate of oxygen evolution in the presence of exogenous electron acceptor 1,4-benzoquinone (BQ). The reaction medium contained 20 mM MES (pH 6.5), 10 mM NaCl, 5 mM $MgCl_2$, 400 mM sucrose and 0.2 mM BQ. The chlorophyll concentration was 25 μg $ml^{-1}$.

### Statistical analysis

The results are mean values from 3 to 5 independent experiments. The statistical differences between the means were determined using a two-tailed paired Student's *t* test. Values of $P < 0.05$ were considered as significant differences among studied plants.

## Results and discussions

The extent of photoinhibition depends on the balance between the damage and recovery processes in the photosynthetic apparatus. Gombos et al. (1994) found that an apparent increase in photoinhibition of photosynthesis in vivo, at low temperature is caused by a decrease in the rate of recovery at low temperature. The study of the light stress on isolated thylakoid membranes at 4 °C should provide clearer picture for the damage occurring in the photosynthetic apparatus during photoinhibition because almost no recovery is carried out in these conditions.

The low temperature chlorophyll fluorescence spectra were used to check energy transfer between two

**Table 1** The influence of high-light treatment on the low temperature fluorescence F685/F735 ratio of thylakoid membranes from: *Chlorotica* XV/1422, Auralia wild type, *Costata* 2/133, Borec wild type and *Coreuleovireus* 2/16

| Time (min) | *Chlorotica* XV/1422 | Auralia wild type | *Costata* 2/133 | Borec wild type | *Coreuleovireus* 2/16 |
|---|---|---|---|---|---|
| 0 | 0.81 ± 0.04 | 0.85 ± 0.05 | 0.87 ± 0.02 | 0.90 ± 0.04 | 1.02 ± 0.02 |
| 30 | 0.80 ± 0.02 | 0.76 ± 0.03 | 0.83 ± 0.02 | 0.88 ± 0.02 | 0.97 ± 0.04 |
| 60 | 0.69 ± 0.03 | 0.74 ± 0.01 | 0.80 ± 0.03 | 0.80 ± 0.01 | 0.90 ± 0.01 |
| 120 | 0.66 ± 0.01 | 0.70 ± 0.02 | 0.72 ± 0.01 | 0.73 ± 0.02 | 0.80 ± 0.02 |

photosystems in photosynthetic apparatus after high-light treatment depending on the lipid composition and LHCII organization. The lateral migration of the main LHCII is thought to be essential for short-term acclimation of the photosynthetic apparatus to high-light (Calberg et al. 1992) and it is important factor for protecting PSII against photoinhibition (Tyystjärvi et al. 1994). The fluorescence emission spectra of five types of thylakoid membranes (before and after light treatment) performed at 77 K exhibit three bands as follow: at 685 nm and at 695 nm related to PSII and one at 735 nm related to PSI (Satoh et al. 1976; Krause and Weis 1991). Analysis of the chlorophyll fluorescence emission spectra showed that F685/F735 ratio (reflecting energy redistribution between two photosystems) decreased in light treated thylakoid membranes in comparison to the control membranes (Table 1). Decrease in this fluorescence ratio after 120 min treatment is about 20 %, which demonstrates that the energy transfer from PSII to PSI increases as a result of the high-light treatment. The results for excitation of Chl *b* are similar to those when Chl *a* is exited (data not shown). The changes in the F685/F735 ratio are almost identical in all studied thylakoid membranes i.e., the increase of the energy transfer to PSI does not depend on LHCII oligomerization and/or amount of anionic lipids (SQDG and PG).

To obtain information about the effect of strong light on PSII activity of studied thylakoid membranes, the oxygen evolution was measured in the presence of exogenous electron acceptor benzoquinone (Fig. 1). Photoinactivation of PSII in all studied pea species increases with an increase of treatment time. The results revealed inhibition of the oxygen evolution after 120 min high-light treatment, depending on the LHCII structural organization (LHCIIo/LHCIIm) and/or lipid composition. The inhibition of PSII activity is smaller for *Chlorotica* XV/1422 (the smallest ratio of LHCIIo/LHCIIm) in comparison to *Coreuleovireus 2/16* (the highest ratio of LHCIIo/LHCIIm).

The presented results suggest that the sensitivity of the photosynthetic apparatus decreases under the high-light irradiation with increase of the oligomerization of LHCII (Fig. 1), which correlates with decreased amount of anionic lipids (Dankov et al., 2009, 2011). In contrast, the earlier observations reported that the rate constant of

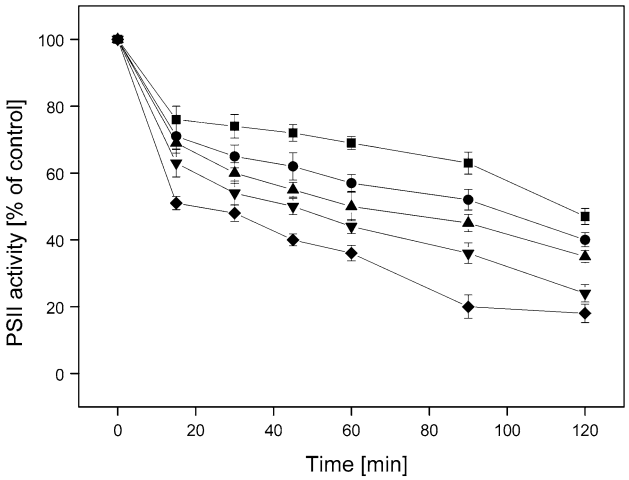


**Fig. 1** Time-course of oxygen evolution in the presence of exogenous electron acceptor BQ in pea thylakoid membranes during the high-light treatment (1,300 μmol $m^{-2}$ $s^{-1}$) at low temperature. Photochemical activity of PSII is given as a % of control. *Coreuleovireus 2/16* (*filled square*), *Costata 2/133* (*filled circle*), Borec wild type (*filled triangle*), Auralia wild type (*filled inverted triangle*) and *Chlorotica XV/1422* (*filled diamond*). Significant differences were registered between *Chlorotica XV/1422* and *Coreuleovireus 2/16* ($P < 0.001$), *Costata 2/133* ($P < 0.001$) Borec wild type ($P < 0.01$)

photoinhibition in vitro does not depend on the antenna size (Tyystjärvi et al. 1994). Considering the following facts: (i) Tyystjärvi et al. (1994) used thylakoid membranes from plants grown at low and high-light intensity, which contain similar lipid class composition (Chapman et al. 1986); (ii) the significant role of SQDG and PG for the organization and stability of the PSII complex (Domonkos et al. 2008; Sato et al. 2003); and (iii) the results in the present study, it could suppose that the amount of the anionic lipids affects the stability of PSII complex to high-light stress.

**Acknowledgments** I would like to thank Prof. A.N. Misra, Central University of Jharkhand, India, for critical readings and useful suggestions that improved the manuscript also I would like to thank to Ts. Markova for the measure of the photosynthetic oxygen evolution and Dr. N. Naydenova, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences for the donation of the seeds of pea chlorophyll mutants. This work was supported by the Bulgarian Academy of Sciences.

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