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ORIGINAL PAPER

Sensitivity of the photosynthetic apparatus to UV-A radiation: role of light-harvesting complex II–photosystem II supercomplex organization

Pavlina I. Ivanova · Anelia G. Dobrikova · Stefka G. Taneva · Emilia L. Apostolova

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Abstract In this work we study the effect of UV-A radiation on the function of the photosynthetic apparatus in thylakoid membranes with different organization of the light-harvesting complex II–photosystem II (LHCII–PSII) supercomplex. Leaves and isolated thylakoid membranes from a number of previously characterized pea species with different LHCII size and organization were subjected to UV-A treatment. A relationship was found between the molecular organization of the LHCII (ratio of the oligomeric to monomeric forms of LHCII) and UV-A-induced changes both in the energy transfer from PSII to PSI and between the chlorophyll–protein complexes within the LHCII–PSII supercomplex. Dependence on the organization of the LHCII was also found with regard to the degree of inhibition of the photosynthetic oxygen evolution. The susceptibility of energy transfer and oxygen evolution to UV-A radiation decreased with increasing LHCII oligomerization when the UV-A treatment was performed on isolated thylakoid membranes, in contrast to the effect observed in thylakoid membranes isolated from pre-irradiated pea leaves. The data suggest that UV-A radiation leads mainly to damage of the PSIIα centers. Comparison of membranes with different organization of their LHCII–PSII supercomplex shows that the oligomeric forms of LHCII play a key role for sensitivity to UV-A radiation of the photosynthetic apparatus.

Abbreviations

A	Initial oxygen burst
BQ	1,4-Benzoquinone
Chl	Chlorophyll
LHCII	Light-harvesting chlorophyll *a/b* protein complex of photosystem II
LHCIIo/m	Oligomeric to monomeric forms of LHCII
OEC	Oxygen evolving complex
PSI	Photosystem I
PSII	Photosystem II
Q_A	Primary quinone electron acceptor of PSII
Q_B	Secondary quinone electron acceptor of PSII
S_i	Redox state *i* of the water oxidizing system
wt	Wild type
Y_3	Amplitude of the oxygen flash yields after third flash

Introduction

The UV-A (320–400 nm) component of sunlight is a significant damaging factor of photosynthesis. The information related to this low-energetic but intense UV-A radiation on plants is limited. UV-A constitutes a major portion of the solar radiation and passes through the stratospheric ozone layer almost unattenuated [1].

In plants and other photosynthetic organisms chlorophylls and other pigments can be destroyed by UV radiation [1]. It has been suggested that UV-B radiation may directly affect the various pigment–protein complexes and the thylakoid membrane ultrastructure. The UV-B damage

S. G. Taneva is Associated member of the Institute of Biophysics, Bulgarian Academy of Sciences.

P. I. Ivanova · A. G. Dobrikova · E. L. Apostolova (✉)
Institute of Biophysics, Bulgarian Academy of Sciences,
Acad.G.Bonchev Str., Bl.21, Sofia 1113, Bulgaria
e-mail: emya@obzor.bio21.bas.bg

S. G. Taneva
Unidad de Biofísica (CSIC/UPV-EHU) y Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad del País Vasco, Bilbao, Spain

to the light-harvesting pigment–protein complexes may significantly exceed that of other proteins [2]. Renger et al. [3] have shown that UV-B radiation may lead to functional disconnection of the light-harvesting chlorophyll *a/b* protein complex (LHCII) from the core complex of photosystem II (PSII) in isolated thylakoid membranes. The structural integrity of chloroplast membranes is altered, from slight swelling and dilation of membranes to physical disruption of the chloroplasts, in plants exposed to UV radiation for various periods of time [4]. However, the structural damage of the chloroplast membranes, although a contributing factor, may not be the only factor causing depressed PSII activity and photosynthetic reaction rates.

Although the damaging efficiency of UV-A is much smaller, it appears to lead to a similar inactivation of the light reactions of photosynthesis as does UV-B [5, 6]. Turcsányi and Vass [7] have reported that the electron transfer is affected by UV-A radiation both at the oxygen-evolving complex (OEC) and at the Q_B-binding site of the PSII complex. Simultaneous monitoring of the damage induced by UV-A and UV-B in both the donor and acceptor sides has shown that the primary lesions occur in the Mn cluster of OEC, followed by a subsequent impairment of the quinone electron acceptors and redox-active tyrosine [5, 8]. The mechanism by which the redox components are impaired is not yet clear.

UV-A irradiation was shown to damage the primary photochemistry of PSII to a larger extent than that of photosystem I (PSI) [9, 10], but the underlying mechanisms are not understood in detail. It was suggested that Q_A and Q_B acceptors are potential targets of UV radiation as a consequence of the damage to D1 and D2 proteins, which form the reaction center of PSII [10–14]. It is also supposed that the UV-A damage has a more pronounced effect on D1 than on D2 [9].

It was shown that different plant types differ in their sensitivity to UV radiation [1]. Moreover, PSIIα in grana domains were found to be more susceptible to UV-B radiation than PSII*β* centers in stroma lamellae [15, 16]. On the other hand, controversial suggestions have been made concerning the dependence of photoinhibition on the size of PSII light-harvesting antennae [17– 19].

The aim of this work was to investigate how the organization of the LHCII–PSII supercomplex affects the susceptibility of plants to UV-A radiation. We used wild-type (wt) pea species (Borec and Auralia) and mutant forms (*Coeruleovireus 2/16*, *Costata 2/133* and *Chlorotica XV/1422)*, as model systems, which differ in amount and degree of oligomerization of LHCII [20, 21]. Our previous investigations [20–22] of these pea thylakoid membranes have shown that: (1) the amount of LHCII is higher for Borec wt and its mutants (*Coeruleovireus 2/16* and *Costata 2/133*) than for Auralia wt and *Chlorotica XV/1422*; (2) the ratio LHCIIo/m of the oligomeric (heterotrimeric, containing three polypeptides: *Lhcb1*, *Lhcb2* and *Lhcb3*) to monomeric forms (built by *Lhcb4*, *Lhcb5* and *Lhcb6*) of LHCII varies in thylakoid membranes from *Chlorotica XV/1422* (LHCIIo/m = 2.45), Auralia wt (LHCIIo/m = 2.82), *Costata 2/133* (LHCIIo/m = 3.34*)*, Borec wt (LHCIIo/m = 4.87) and *Coeruleovireus 2/16* (LHCIIo/m = 6.62); (iii) the degree of LHCII oligomerization in the studied pea species correlates with the ratio of the functionally active PSIIα to PSII*β* centers.

In the present investigation we show that the effect of UV-A on the photosynthetic oxygen evolution and the 77K chlorophyll fluorescent characteristics of the thylakoid membranes strongly depend on their ultrastructure.

Materials and methods

Preparation of thylakoid membranes

Thylakoid membranes were isolated from *Pisum sativum* L. cv. Borec, its mutants *Coeruleovireus 2/16* and *Costata 2/133*, and *Pisum sativum* L. cv. Auralia and its mutant *Chlorotica XV/1422* as described in Harrison and Melis [23]. The total chlorophyll concentration was determined by the method of Lichtenthaler [24].

The plants were grown at controlled conditions under 250 μmol photons m^{-2} s^{-1}, with 16 h light/8 h dark photoperiod. The mutants used in this study are well defined and stable. In each new series of studies of these mutants and wild types, we checked their properties. Their membranes did not reveal any differences in physico-chemical characteristics and functions.

Low temperature (77K) chlorophyll fluorescence

Low temperature (77K) 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 $MgCl_2$, 330 mM sucrose at chlorophyll concentration 20 μg ml^{-1}. The samples were quickly frozen by plunging them in liquid nitrogen. Fluorescence spectra were recorded from 600 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*).

The 77K chlorophyll fluorescence emission spectra of thylakoid membranes exhibit three bands: 685, 695 and 735 nm. The emission bands at 685 and 695 nm are related

to PSII, and the one at 735 nm to PSI [25, 26]. To assess the effects of UV-A radiation on the energy transfer between the chlorophyll-protein complexes of the thylakoid membranes we used the chlorophyll emission ratios F_{735}/F_{685} (for estimation of the energy redistribution between the two photosystems) and F_{695}/F_{685} (for the energy transfer between chlorophyll–protein complexes in the LHCII–PSII supercomplex).

Oxygen evolution measurements

Oxygen flash yields and initial oxygen burst were measured using a custom-built polarographic oxygen rate electrode as described by Zeinalov [27]. Thylakoid membranes were suspended in a medium without artificial electron acceptor containing: 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose. The chlorophyll concentration was 150 μg ml^{-1} in 100 μl sample volumes and formed a layer of 2 mm height. Samples were pre-illuminated with ca. 25 flashes and then dark adapted for 5 min before measurements. Oxygen flash yields were induced by saturating (4 J) and short ($t_{1/2}$ = 10 μs) periodic flash sequences with 650 ms dark spacing between the flashes. The initial oxygen burst was recorded after irradiation with continuous white light (450 μmol photons m^{-2} s^{-1}). The induction curves after oxygen burst exhibit biphasic exponential decay. Deconvolution of the oxygen burst decay was performed by fitting of the function with two exponential components: $A_1e^{-(tk1)} + A_2e^{-(tk2)}$, where A_1, A_2 are amplitudes and k_1, k_2 are rate constants of the fast and slow components, respectively.

The initial S_0–S_1 state distribution in the dark was determined by the least square deviations fitting to the theoretically calculated yields according to the model of Kok et al. [28] with the experimentally obtained oxygen flash yields.

Photochemical activity of PSII

Steady-state rate of oxygen evolution (photochemical activity of PSII) was measured polarographically with a Clark-type electrode (Model DW1, Hansatech, Instruments Ltd. King's Lynn, Norfolk) in a temperature-controlled cuvette at 20°C, using saturated white (achromatic) light. The PSII activity was measured by the rate of oxygen evolution in the presence of the exogenous electron acceptor 1,4-benzoquinone (BQ). The reaction medium contained 20 mM MES (pH 6.5), 10 mM NaCl, 5 mM $MgCl_2$, 330 mM sucrose and 0.2 mM BQ. The chlorophyll concentration was 25 μg ml^{-1}.

UV-A treatment of leaves and isolated thylakoid membranes

Leaves or thylakoid membranes were illuminated in a petri dish at 20°C with maximal UV-A emission at 365 nm from a Cole-Parmer ultraviolet lamp (Model 3UV-34) equipped with a suitable filter. The range of the emission of the UV lamp is from 340 to 400 nm. The intensity of radiation, measured with a radiometer/photometer IL 1400A, was 15 W m^{-2}.

Thylakoid membranes during the illumination for 0–180 min were suspended in a buffer containing 40 mM HEPES (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose at a chlorophyll concentration 500 μg ml^{-1}, forming a 1 mm thin suspension layer at continuous stirring.

Leaves were illuminated for 180 min. Thylakoid membranes isolated from UV-treated or untreated leaves were suspended in the medium desired for the different measurements. All samples were dark-adapted for 15–30 min before measurements.

The control samples (non-irradiated) were kept in the dark.

Statistical analysis

The results were averaged from three to five independent experiments. The experiments were performed in triplicate. 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 significantly different between the studied pea thylakoid membranes.

Results

UV-A effect on the low temperature (77K) chlorophyll fluorescence

Analysis of the fluorescence emission spectra of the thylakoid membranes shows that both the F_{735}/F_{685} ratio (reflecting the energy redistribution between the two photosystems) and the F_{695}/F_{685} ratio (related to the energy transfer between chlorophyll–protein complexes in the LHCII–PSII supercomplex) increase with increasing duration of UV-A treatment of the isolated thylakoid membranes (Tables 1 and 2). The changes in the F_{735}/F_{685} ratio are negligible in all studied thylakoid membranes during 60 min UV-A exposure. The largest changes in the emission ratios are observed for the mutant *Chlorotica XV/1422* (Tables 1 and 2), which has the smallest LHCIIo/m ratio. For this mutant the F_{735}/F_{685} ratio increases (in comparison to the control thylakoid membranes) by about 65 and 83% after 180 min UV-A irradiation when Chl *a* (Table 1) and Chl *b* (Table 2) were excited, respectively. The increase of F_{735}/F_{685} ratio is not more than 48% (exc. Chl *a*) and 58% (exc. Chl *b*) for the other pea species. Generally, the changes in both F_{735}/F_{685} and F_{695}/F_{685} ratios after UV-A treatment are larger after excitation of Chl *b* than of Chl *a*.

Table 1 Low temperature (77K) chlorophyll fluorescence emission ratios, F_{735}/F_{685} and F_{695}/F_{685}, at excitation of Chl *a* (436 nm) after UV-A irradiation

UV treatment on:	Thylakoid membranes						Leaves
Irradiation time (min)	0	30	60	90	120	180	180
Chlorotica XV/1422							
F_{735}/F_{685}	1.33	1.27	1.56	1.79	1.89	2.20	1.35
F_{695}/F_{685}	0.72	0.76	0.86	0.91	0.94	1.12	0.71
Auralia wt							
F_{735}/F_{685}	1.28	1.32	1.37	1.4	1.59	1.89*	1.32
F_{695}/F_{685}	0.75	0.76	0.77	0.79	0.82	0.86***	0.82
Costata 2/133							
F_{735}/F_{685}	1.23	1.42	1.43	1.64	1.68	1.79*	1.34
F_{695}/F_{685}	0.76	0.75	0.73	0.76	0.79	0.88***	0.78
Borec wt							
F_{735}/F_{685}	1.11	1.20	1.40	1.38	1.39	1.50**	1.12
F_{695}/F_{685}	0.76	0.77	0.78	0.81	0.84	0.99**	0.80
Coeruleovireus 2/16							
F_{735}/F_{685}	0.98	1.10	1.11	1.25	1.35	1.40**	1.10*
F_{695}/F_{685}	0.73	0.75	0.76	0.77	0.78	0.85***	0.86*

The treatment was made on isolated thylakoid membranes (for different times) and leaves (for 180 min) from *Chlorotica XV/1422*, Auralia wt, *Costata 2/133*, Borec wt and *Coreuleovireus 2/16*. Average data are from three to four independent experiments. The standard errors range from ±0.03 to ±0.07 for the ratio F_{735}/F_{685} and from ±0.01 to ±0.05 for the ratio F_{695}/F_{685}. *Asterisks* mark significant differences in the UV-induced changes of *Chlorotica XV/1422* in comparison to all other pea species (*$P < 0.05$, **$P < 0.01$)

Table 2 Low temperature (77K) chlorophyll fluorescence emission ratios, F_{735}/F_{685} and F_{695}/F_{685}, at excitation of Chl *b* (472 nm) after UV-A irradiation

UV treatment on:	Thylakoid membranes						Leaves
Irradiation time (min)	0	30	60	90	120	180	180
Chlorotica XV/1422							
F_{735}/F_{685}	1.15	1.13	1.29	1.53	1.69	2.11	1.12
F_{695}/F_{685}	0.74	0.84	0.93	1.03	1.05	1.31	0.73
Auralia wt							
F_{735}/F_{685}	1.15	1.24	1.24	1.31	1.31	1.76**	1.18
F_{695}/F_{685}	0.82	0.85	0.89	0.90	0.91	1.04***	0.84
Costata 2/133							
F_{735}/F_{685}	0.94	1.12	1.14	1.29	1.31	1.49**	1.06*
F_{695}/F_{685}	0.89	0.87	0.86	0.88	0.95	1.05***	0.90
Borec wt							
F_{735}/F_{685}	0.82	0.92	1.07	1.04	1.04	1.20**	0.88*
F_{695}/F_{685}	0.92	0.93	1.02	1.03	1.06	1.26**	1.03*
Coeruleovireus 2/16							
F_{735}/F_{685}	0.78	0.89	0.93	1.03	1.03	1.15**	0.94**
F_{695}/F_{685}	0.99	1.02	1.05	1.06	1.13	1.20***	1.08*

The treatment was made on isolated thylakoid membranes (for different times) and leaves (for 180 min) from *Chlorotica XV/1422*, Auralia wt, *Costata 2/133*, Borec wt and *Coreuleovireus 2/16*. Average data from three to four independent experiments are presented. The standard errors range from ±0.03 to ±0.07 for the ratio F_{735}/F_{685} and from ±0.01 to ±0.05 for the ratio F_{695}/F_{685}. *Asterisks* mark significant differences in the UV-induced changes of *Chlorotica XV/1422* in comparison to all other pea species (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

The alterations observed in the energy transfer between the chlorophyll–protein complexes for leaves exposed to 180 min UV-A radiation were significantly smaller than those for irradiated thylakoid membranes (Tables 1 and 2). In contrast to the changes in UV-A irradiated thylakoid membranes, the UV-A effect on the energy transfer was larger for pea species with higher LHCIIo/m ratios when the membranes were isolated from pre-irradiated leaves. The increase of the fluorescence ratio F_{735}/F_{685} is about 20% and that of F_{695}/F_{685} about 12% for *Coeruleovireus 2/16* after excitation of Chl *b* (Table 2).

UV-A effect on oxygen evolution in presence of an exogenous electron acceptor

The inhibition of the steady-state oxygen evolution ($H_2O \rightarrow BQ$), which reflects the decrease of electron transport through PSII, after UV-A treatment of the thylakoid membranes is shown in Fig. 1. The PSII-mediated electron transport is reduced by 58% to 88%, after 180 min UV-A treatment of the different pea thylakoid membranes and the reduction strongly depends on the amount of LHCII and its oligomerization state. The PSII activity is inhibited to a lower degree in thylakoid membranes from Borec wt and its mutants, which have more LHCII and a higher LHCIIo/m ratio, than Auralia wt and *Chlorotica XV/1422*.

Similar to the UV-A radiation effect on the energy transfer, the PSII-mediated electron transport was inhibited less when UV-A treatment (180 min) was performed on leaves rather than on isolated thylakoid membranes. In this case, the inhibition is 20% for Auralia wt and *Chlorotica XV/1422*, while for Borec wt and its mutants it is about 50% (data not shown).

UV-A effect on the oxygen induction curves

Oxygen induction curves under continuous illumination were measured in control (non-irradiated) and UV-A-treated thylakoid membranes from the different pea species. The induction curves of the mutants *Coeruleovireus 2/16* and *Chlorotica XV/1422*, which possess the highest and the smallest LHCIIo/m ratio, respectively, are presented in Fig. 2. The amplitude of the initial oxygen burst (*A*) and the area under the curve (representing the oxygen volume

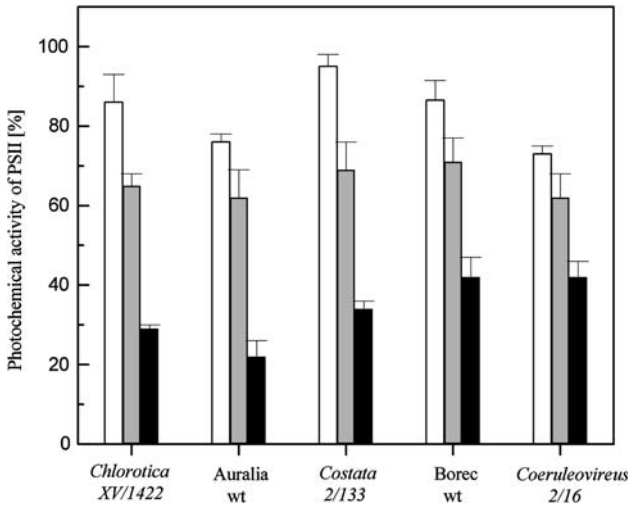

Fig. 1 Effect of UV-A radiation on the steady-state oxygen evolution (PSII-mediated electron transport) of the thylakoid membranes from the pea species *Chlorotica XV/1422*, Auralia wt, *Costata 2/133*, Borec wt and *Coreuleovireus 2/16*. Isolated thylakoid membranes were irradiated for 30 min (*white bars*), 90 min (*gray bars*) and 180 min (*black bars*). The values of the oxygen evolution are normalized to the value in non-irradiated control thylakoid membranes. Mean values ± SD were determined from four to five independent experiments. Significant statistical differences are registered when *Chlorotica XV/1422* and Auralia wt are compared with *Coreuleovireus 2/16* ($P < 0.05$). Statistical differences are registered at comparison of the mutant *Chlorotica XV/1422* and Auralia wt with *Costata 2/133* and Borec wt ($P < 0.1$)

evolved) depend on the number of functionally active PSII centers [29]. The oxygen burst decay of the untreated membranes shows biphasic kinetics (see Fig. 2). The ratio of the amplitudes of the fast and slow components (A_1/A_2) corresponds to the proportion of oxygen production by non-cooperative (A_1) and cooperative (A_2) mechanisms (the ratio of the functionally active PSIIα to PSIIβ centers) [22, 29]. Data show that 120 min UV-A treatment induces a substantial reduction of the functionally active PSII centers (Fig. 2a, b insets). In addition, the results reveal a decrease and smoothing of the initial amplitude of the oxygen burst after UV-A treatment due to the drastic reduction (Fig. 2a inset) or complete loss (Fig. 2b inset) of the fast A_1 component. This hints at UV-induced inhibition of PSIIα centers evolving oxygen through non-cooperative Kok's mechanisms.

The parameters obtained from the analysis of the induction curves of all studied pea species during UV-A treatment, the amplitude of the initial oxygen burst (A) and the ratio A_1/A_2, are summarized on Table 3. The oxygen burst under continuous illumination is reduced to a different extent during UV-A irradiation depending on the irradiation time. The largest inhibition, about 85%, of the initial oxygen burst, is observed after 120 min treatment of *Chlorotica XV/1422*, which is characterized by the lowest oligomerization of LHCII proteins, whereas the smallest inhibition, about 56%, of the oxygen burst is found for *Coeruleovireus 2/16* which has the highest LHCIIo/m ratio (Table 3). The ratio A_1/A_2 strongly decreases during prolonged (120 min) UV-A treatment of thylakoid membranes from *Coeruleovireus 2/16*; however, the fast A_1 component of the oxygen burst decay is not lost, whereas for *Chlorotica XV/1422* the fast A_1 component disappears after 45 min of UV-A irradiation (Table 3). This might indicate a destruction of the grana domains.

Hence, the decrease of A_1/A_2 ratios after UV-A treatment depends on the amount and oligomerization of LHCII in thylakoid membranes. The fastest reduction of the parameter A_1/A_2 is observed for *Chlorotica XV/1422* (Table 3). It is reasonable to attribute this reduction to an inhibition of

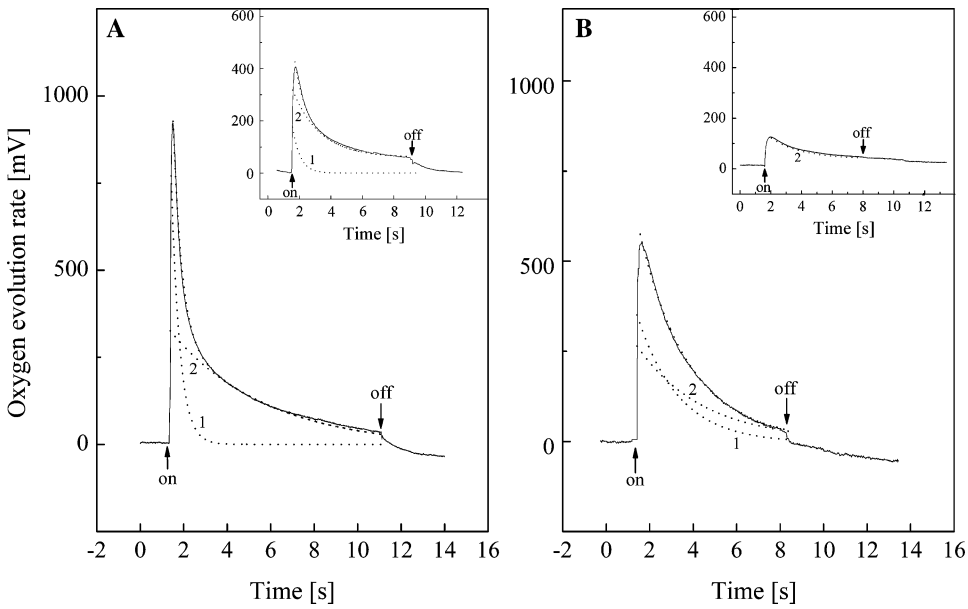

Fig. 2 Initial oxygen burst kinetics under continuous irradiation (450 µmol photons m^{-2} s^{-1}) of control thylakoid membranes from pea mutants: **a** *Coreuleovireus 2/16* and **b** *Chlorotica XV/1422*. Two exponential components (*dotted lines*) are used to the deconvolution of the oxygen burst decay: *line 1* fast component (A_1) and *line 2* slow component (A_2) (for details see "Materials and methods"). The *insets* show the effect of 120 min UV-A irradiation on the decay kinetics of initial oxygen burst of the two studied mutants. Time constant of the electrode is less than 2 ms. Polarograph sensitivity is 1.5 V μA^{-1}

Table 3 Effect of UV-A radiation on the parameters of the initial oxygen burst and flash oxygen yields of the investigated pea thylakoid membranes

UV treatment on:	Thylakoid membranes						Leaves
Irradiation time (min)	0	15	30	45	60	120	180
Chlorotica XV/1422							
A (%)	100	87	74	55	36	15	75
A_1/A_2	0.61	0.57	0.52	–	–	–	0.49
Y_3 (%)	100	65	60	51	40	9	73
S_0 (%)	16	19	21	23	27	38	20
Auralia wt							
A (%)	100	95	85	82	79	35	80
A_1/A_2	1.38	1.29	1.11	0.99	0.87	–	1.13
Y_3 (%)	100	83	70	63	46	23	80
S_0 (%)	17	19	21	23	28	39	20
Costata 2/133							
A (%)	100	84	73	64	63	36	67
A_1/A_2	2.28	1.71	1.64	1.40	1.27	–	1.39
Y_3 (%)	100	101	65	43	40	21	69
S_0 (%)	20	22	24	26	28	30	28
Borec wt							
A (%)	100	97	89	80	72	38	56
A_1/A_2	2.50	2.05	1.69	1.37	1.17	–	1.45
Y_3 (%)	100	82	68	67	47	24	57
S_0 (%)	21	22	24	25	26	29	25
Coeruleovireus 2/16							
A (%)	100	94	75	63	60	44	63
A_1/A_2	2.71	2.38	2.12	1.91	1.44	0.89	1.76
Y_3 (%)	100	70	53	40	30	20	70
S_0 (%)	25	27	28	31	33	38	34

– represents mono-exponential decay ($A_1 = 0$)

A the initial oxygen burst amplitude; A_1/A_2 ratio between the amplitudes of the fast (A_1) and the slow (A_2) components; Y_3 the oxygen yield of the third flash; S_0 the populations of PSII centers in S_0 state in the dark (S_0 [%] = 100 × S_0 /(S_0 + S_1)). UV-A treatment was made on thylakoid membranes for different periods of time and on leaves for 180 min. Significant differences between the parameters (after 120 min irradiation of the thylakoid membranes and 180 min irradiation of leaves) were indicated at pairwise comparisons between *Chlorotica XV/1422* and Auralia wt ($P < 0.01$) with Borec wt and its mutants ($P < 0.01$). Between *Chlorotica XV/1422* and *Coeruleovireus 2/16* ($P < 0.001$). A and Y_3 for different pea species are represented as percentage of the values for non-irradiated control membranes, considered as 100%. A_1 and A_2 components are obtained from deconvolution of the initial oxygen burst decay (for details see Fig. 2 and "Materials and methods")

predominantly PSIIα centers that evolve oxygen through non-cooperative mechanism and give rise to the fast A_1 component. This is in agreement with other observations hinting at a higher UV sensitivity of PSIIα compared to PSIIβ [15, 16]. It is worth mentioning that thylakoid membranes from *Chlorotica XV/1422* have the lowest amount of PSIIα centers [22].

The induction curves of thylakoid membranes isolated from leaves irradiated with UV-A for 3 h also show a decrease of the functionally active PSII centers and the ratio of the active PSIIα to PSIIβ centres (A_1/A_2) (Table 3). It is seen that in this case the UV treatment leads to more significant damage of the functionally active PSIIα centers in the membranes, which have a higher amount and oligomerization state of LHCII and, correspondingly, a higher degree of membrane stacking (Borec wt and its mutants).

UV-A effect on the oxygen flash yields

Our data show that the UV-A radiation induces a reduction in the oxygen flash yields that depends on the organization of the PSII complex. Flash-induced oxygen patterns for the control and UV-A treated isolated thylakoid membranes of Auralia wt and its mutant *Chlorotica XV/1422* are shown in Fig. 3. Characteristic oscillations with a periodicity of four are observed for both the control and short-time irradiated membranes. The oxygen yields gradually decrease with increasing irradiation time (Fig. 3). The amplitude of the oxygen flash yields after the third flash (Y_3) was used to estimate the UV-A-induced inhibition of the active PSII centers that evolve oxygen by non-cooperative mechanism (PSIIα). The values of Y_3 are given in Table 3. The results show a strong reduction of the flash-induced oxygen yields after 120 min of UV treatment. These observations are in close agreement with previously reported data [9], which suggested that UV-A radiation induced a substantial loss of the flash-induced oxygen yields. More significant inactivation of the oxygen yields (Y_3) is observed for *Chlorotica XV/1422* (Table 3, Fig. 3b) which, as mentioned above, possess the smallest LHCII size and PSIIα/PSIIβ ratio and, subsequently, the lowest degree of grana stacking as compared to the other pea species [22]. The drastic decrease in the flash-induced oxygen yields after UV radiation could be a consequence of the destruction of the grana domains.

The PSII centers in the initial S_0 state increase during UV-A irradiation (Table 3). Similar UV-A-induced changes in spinach thylakoid membranes were reported by Turcsányi and Vass [9]. A stronger effect on S_0–S_1 distribution in the dark is registered for *Chlorotica XV/1422* and Auralia wt than for the other pea species (Table 3). Untreated membranes of both *Chlorotica XV/1422* and Auralia wt have a smaller amount of PSII centers in S_0 state (Table 3), lower LHCIIo/m ratio and smaller amount of LHCII per PSII reaction center than the rest of the investigated membranes [22].

A reduction of the active oxygen-evolving centers and an increase of the S_0 population in darkness are also observed when thylakoid membranes were isolated from UV-A-irradiated leaves (Table 3). In contrast to the effects observed

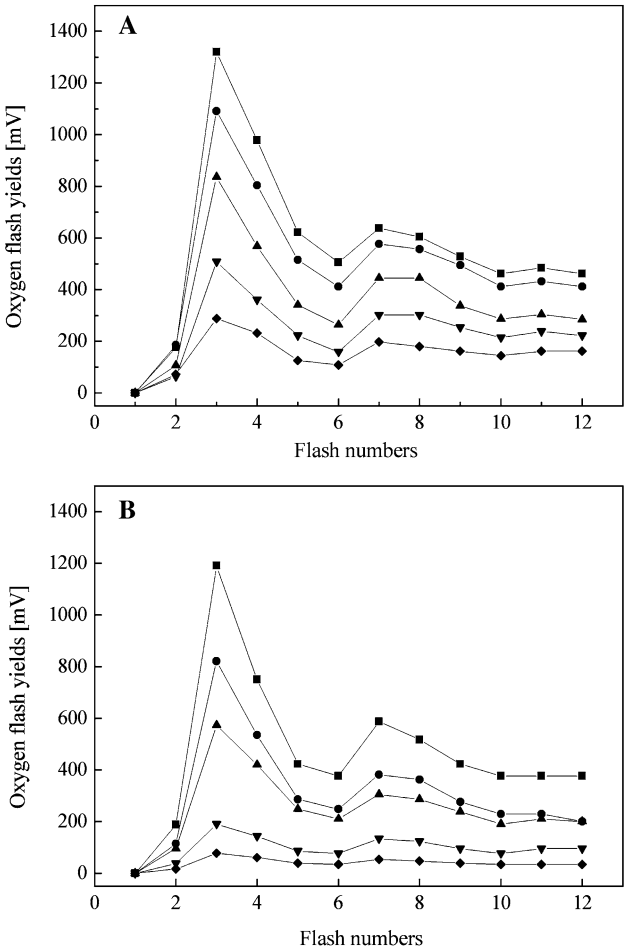

Fig. 3 Effect of UV-A radiation on the oscillation patterns of the oxygen flash yields as a function of the flash number in dark-adapted (for 5 min) thylakoid membranes from Auralia wt (*A*) and *Chlorotica XV/1422* mutant (*B*). Control (*filled square*) and UV-A treated membranes for 15 min (*filled circle*), 45 min (*filled triangle*), 90 min (*filled inverted triangle*) and 120 min (*filled diamond*). The treatment was made on isolated thylakoid membranes. Polarograph sensitivity is 3 V μA^{-1}

for UV-A-treated thylakoid membranes, these effects are more pronounced for pea species with a higher LHCIIo/m ratio (Borec wt and its mutants *Costata 2/133* and *Coeruleovireus 2/16*) than for those with a lower degree of LHCII oligomerization (Auralia wt and *Chlorotica XV/1422*).

Discussion

It is known that the primary target of UV-A radiation in thylakoid membranes is the PSII complex [5, 8]. Several UV sensitive sites are supposed to exist in this complex, including the redox-active tyrosine, the Mn cluster on the donor side and the plastosemiquinones on the acceptor side [5, 8, 30]. The primary site of UV-A radiation damage is thought to be the catalytic Mn cluster of the oxygen evolving system [5]. The inactivation of the electron transport between the Mn cluster and the tyrosine electron donors was proposed to be the immediate cause for the loss of O_2 evolution by UV radiation [5]. It is also known that UV-A treatment leads to damage of D1 and D2 protein subunits from the reaction center of PSII [10–14, 31], as well as to destruction of Q_A and Q_B acceptor molecules and modification of their surroundings [5, 9, 10]. These structural changes in the photosynthetic apparatus affect the energy distribution between the two photosystems (ratio F_{735}/F_{685}) and the energy transfer between the pigment–protein complexes within the PSII multi-component complex (ratio F_{695}/F_{685}). The more pronounced changes after excitation of Chl *b* (472 nm) (Table 2) than of Chl *a* (436 nm) (Table 1), indicate that UV-A radiation has an effect on the energy transfer between Chl *b* and Chl *a*.

The UV-induced changes observed in the energy transfer between chlorophyll–protein complexes in the LHCII–PSII supercomplex might be attributed to disconnection of LHCII and PSII core complex and/or to damage of LHCII. The increase in the energy transfer to PSI, on the other hand, can be associated with an increased lateral mobility of LHCII complex as a result of UV-induced modification of the photosynthetic apparatus. The UV-induced changes in the energy distribution depend on the amount of LHCII oligomeric forms (Tables 1 and 2). Therefore, a distinct relationship between the LHCII organization and the UV-A effect on the energy transfer was found that depended on whether the irradiation was performed on isolated thylakoid membranes or on leaves.

The inhibition of the oxygen yields and the increase of the S_0 populations after UV-A treatment could be attributed to structural changes in the OEC and modification of its function in the still active centers. Nedunchezhian and Kulandaivelu [32] found that UV-B treatment of chloroplasts caused an inhibition of the PSII activity due to a loss of 23 and 33 kDa extrinsic polypeptides. A possible reason for the inactivation of the Mn cluster of the OEC might be the separation of the 33 kDa protein, which maintains the functional conformation of the catalytic Mn cluster. The protein environment in the vicinity of the Mn cluster may also be damaged by UV radiation due to production of highly reactive radicals [8].

On the other hand, it has been proposed that the primary reason for the UV-induced inhibition of oxygen evolution is the absorption of UV light by Mn ions in Mn (III) and Mn (IV) oxidation states [6]. The oxidation state of the Mn clusters in S_0 (Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{4+}) is lower by one oxidizing equivalent than in S_1 (Mn^{3+}, Mn^{3+}, Mn^{4+}, Mn^{4+}) [33]. Thus, higher amount of Mn ions in Mn (III) oxidation state (increased PSII centers in S_1 state), as observed for control membranes from *Chlorotica XV/1422* and Auralia wt (Table 3), is one of the possible reasons for the higher sensitivity of the photosynthetic apparatus to UV radiation

in these membranes as compared to membranes from Borec and its mutants.

A comparison between the UV-induced inhibition of the oxygen evolution in the presence or absence of an exogenous acceptor after in vitro treatment shows that thylakoid membranes with higher LHCIIo/m ratio and higher degree of grana stacks exhibit a lower sensitivity to UV radiation than those with lower LHCIIo/m ratio and degree of grana stacks (Fig. 1 and Table 3). This may be linked to the ability of non-functional PSII centers to protect the remaining, still functionally active centers from UV-A damage, as was shown to occur in stacked grana domains during prolonged illumination with high light intensity [34]. We suggest that this is the other explanation for the enhanced UV-A resistance of the photosynthetic apparatus with increasing amount of LHCII oligomeric forms.

The changes in A_1/A_2 after UV-A irradiation (Table 3) indicate that the ratio of the functionally active PSIIα to PSIIβ centers decreases and/or that the centers evolving oxygen through non-cooperative Kok's mechanisms (characterized by amplitude A_1) are damaged as a result of higher susceptibility of the PSIIα subpopulation to UV radiation. The variation in the kinetics of the initial oxygen evolution burst after UV-A irradiation might be due to conformational changes on the acceptor side of PSII and modification of the interaction between Q_B and plastoquinone molecules. Alterations of the acceptor side of PSII and/or changes in the OEC have an influence on the affinity or accessibility of the BQ to Q_B and lead to inhibition of the PSII-mediated electron transport.

The presented data for UV-A radiation effects on the photosynthetic apparatus demonstrate: (1) an increase of the energy transfer from PSII to PSI, (2) modification of the energy transfer between the pigment–protein complexes within the LHCII–PSII supercomplex, (3) an inhibition of the PSII-mediated electron transport ($H_2O \rightarrow BQ$), (4) an increase of the S_0 populations of PSII centers in darkness and (5) an inhibition of the flash-induced oxygen yields, as well as of the fast component of the initial oxygen burst under continuous illumination, due to the inactivation of functionally active PSIIα centers in grana domain, which are characterized by large antenna size.

These UV-A induced effects depend on the degree of the LHCII oligomerization (LHCIIo/m ratio), the ratio of the functionally active PSIIα/PSIIβ centers and the amount of the PSII centers in S_0 state in the studied thylakoid membranes. Our data reveal different relationships between the LHCII–PSII organization and the UV-A-induced damage of PSII photochemistry depending on whether isolated thylakoids or intact leaves were irradiated (Fig. 4). Pea thylakoid membranes with a higher LHCIIo/m ratio and, subsequently, a higher degree of grana stacks show a lower sensitivity to UV-A after in vitro treatment (Fig. 4), which may be related to protection of some PSII functional centers by non-functional ones and/or decrease of D1 protein degradation.

The better tolerance to UV-A radiation of the photosynthetic apparatus with smaller LHCIIo/m ratio observed when intact leaves were treated, may be due to an active repair process and/or more powerful protective mechanisms in vivo (Fig. 4). The rate of D1 protein degradation and synthesis is extremely well coordinated in higher plant leaves. Anderson and Aro [34] suggested that D1 degradation plays a crucial role in the regulation of D1 protein turnover in vivo. It is also known that UV-B radiation leads to changes in the degradation and turnover rate of D1 polypeptide [11]. Having in mind that in higher plant thylakoids the photosystems are spatially separated and that damage and synthesis of D1 protein must occur in different membrane domains, it may be supposed that a lower degree of LHCII oligomerization and increased amount of nonappresed thylakoid membranes facilitate the repair process.

We found a relationship between the amount of oligomeric forms of LHCII and UV-A induced changes of the photosynthetic apparatus. An increased antenna size and higher ratio of the oligometic to monomeric forms of LHCII lead to lower UV-A sensitivity of the thylakoid membranes during in vitro treatment and higher sensitivity during in vivo treatment in the studied pea species (Fig. 4). Knowledge of this relationship between the organization of LHCII–PSII supercomplex and the UV-radiation resistance of the photosynthetic apparatus may be useful for genetic

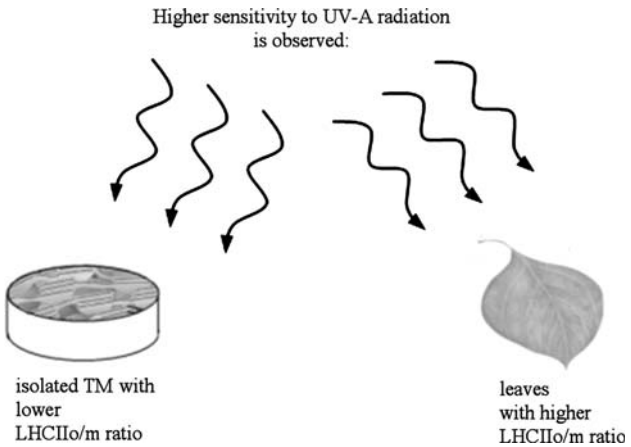

Fig. 4 Schematic presentation of UV-A sensitivity of photosynthetic apparatus depending on LHCII–PSII supercomplex organization. The opposite effects of the oligomerization of the LHCII on the UV-A tolerance of photosynthetic apparatus when isolated thylakoid membranes (*TM*) or leaves were irradiated is seen. The UV-A-induced changes in the energy transfer and the photosynthetic oxygen evolution of pea species with lower LHCIIo/m ratio (i.e., smaller PSIIα/PSIIβ ratio and grana stacks) increase at in vitro treatment (*left*) and decrease at in vivo treatment (*right*)

engineering of plant species more tolerant to the UV-A radiation.

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