Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 261–271
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbabio

A polyamine- and LHCII protease activity-based mechanism regulates the plasticity and adaptation status of the photosynthetic apparatus

Eleni Navakoudis [a,c], Katerina Vrentzou [a], Kiriakos Kotzabasis [a,b,*]

[a] Department of Biology, University of Crete, P.O. Box 2208, 71409 Heraklion, Crete, Greece
[b] Department of Botany, Natural History Museum of Crete, Heraklion, Crete, Greece
[c] Technological Educational Institute, School of Agricultural Technology, Stavromenos, Heraklion, Crete, Greece

Received 2 July 2006; received in revised form 22 January 2007; accepted 8 February 2007
Available online 20 February 2007

Abstract

In the present study we aim to dissect the basis of the polyamine mode of action in the structure and function of the photosynthetic apparatus. Although the modulating effects of polyamines in photosynthesis have been reported since long [K. Kotzabasis, A role for chloroplast-associated polyamines? Bot. Acta 109 (1996) 5–7], the underlying mechanisms remained until today largely unknown. The diamine putrescine was employed in this study, by being externally added to *Scenedesmus obliquus* cultures acclimated to either low or high light conditions. The results revealed the high efficiency by which putrescine can alter the levels of the major photosynthetic complexes in a concerted manner inducing an overall structure and function of the photosynthetic apparatus similar to that under higher light conditions. The revealed mechanism for this phenomenon involves alterations in the level of the polyamines putrescine and spermine which are bound to the photosynthetic complexes, mainly to the LHCII oligomeric and monomeric forms. *In vitro* studies point out to a direct impact of the polyamines on the autoproteolytic degradation of LHCII. Concomitantly to the reduction of the LHCII size, exogenously supplied putrescine, induces the reaction centers' density and thus the photosynthetic apparatus is adjusted as if it was adapted to higher light conditions. Thus polyamines, through LHCII, play a crucial role in the regulation of the photosynthetic apparatus' photoadaptation. The protective role of polyamines on the photosynthetic apparatus under various environmental stresses is also discussed in correlation to this phenomenon.

Keywords: Photosynthetic apparatus; Polyamine; Photoadaptation; Environmental stress; Protease activity; LHCII

1. Introduction

Understanding the environment is crucial for the adaptation of plants to different conditions. As plants do not have the ability of kinesis they are forced to confront many environmental changes (e.g. light intensity and quality, water and mineral deficiency, exposure to various pollutants and temperatures and CO_2 fluctuations) which in turn cause radical changes in the structure and function of the photosynthetic apparatus [2]. Today it is generally accepted that the adaptation of the photosynthetic apparatus to various stimuli is regulated by a common mechanism which is probably activated by various metabolic signals and not by a specific receptor [3–5].

Photosynthetic organisms respond to variations in both the spectral quality and the intensity of light by adjusting the composition and structure of the photosynthetic apparatus. Such adjustments include alterations in the photosystem stoichiometry as well as in the overall abundance of the accessory light-harvesting proteins associated with each photosystem, mainly with PSII. The adaptation of the photosynthetic apparatus to low and high light intensities is a well-documented phenomenon in both higher plants [6] and green algae [7–10].

Abbreviations: Chl, chlorophyll; 1,4-DB, 1,4-diamino-2-butanone; LHCII, light harvesting complex of PSII; PCV, packed cell volume; PS I, photosystem I; PS II, photosystem II; Put, putrescine; Spd, spermidine; Spm, spermine; CPs, core proteins of PS I and II (CPIa and CPa); CPIa, core protein complex of PSI with LHCI; CPa, core protein complex of PSII; HL, high light conditions; LL, low light conditions

* Corresponding author. Department of Biology, University of Crete, P.O. Box 2208, 71409 Heraklion, Crete, Greece. Tel.: +30 2810 394059; fax: +30 2810 394408.
E-mail address: kotzab@biology.uoc.gr (K. Kotzabasis).

0005-2728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2007.02.008

The main features of adaptation from low light (LL) to higher light (HL) conditions involve increases in the levels of the PSII complex, cytochrome b_6f complex, ATP synthase and components of the Calvin cycle, especially of ribulose-1,6-biphosphate carbolylase/oxygenase (Rubisco), accompanied by reductions in the levels of the major chlorophyll a/b-binding light-harvesting complexes associated with PSII (LHCII). These changes lead to an increased capacity for oxygen evolution, electron transport and CO_2 consumption and also to higher chlorophyll a/b (Chl a/b) ratio. Adaptation to low light leads to opposite changes [6]. To accomplish the above changes a de novo synthesis of photosynthetic components (both in the nucleus and in the chloroplast) but also specific degradations under a strictly regulated mechanism have to take place.

PSII is surrounded by its light-harvesting antenna which is comprised of the inner minor antenna complex (built by CP24, CP26 and CP29, coded by the genes lhcb4, 5 and 6) and the outer major antenna complex LHCII. The structure and function of the LHCII has been studied extensively. Its role is not restricted to the capture of photons but it also functions in the protection of the photosynthetic apparatus against excessive energy flow. This is accomplished by its capability to dissipate the excess energy by a mechanism called non-photochemical quenching, which is activated in the timescale of minutes [11–15]. LHCII is composed of three polypeptides, termed Lhcb1, 2 and 3, which form homo- or hetero-trimers. Seven to eight trimers are aggregated around each PSII [16–18]. LHCII also exists in its monomeric form deriving from trimers that have been subject of proteolytic degradation [19,20].

There are also numerous reports establishing the occurrence of polyamines in the photosynthetic apparatus and their role as modulators of its functioning. It is hypothesized so far that polyamines play only a passive role by stabilizing protein structure or by supporting the formation of more complex aggregates like the LHCII [21–24]. There are also data that strongly support the hypothesis that polyamines play a more complex role in the regulation of structure and function of the photosynthetic apparatus [1,25–27]. Also, previously published results [28–32] indicate that many environmental conditions (stressors) induce changes in the structure and function of the photosynthetic apparatus that resemble its adaptation to either low or high light conditions. Interestingly, exogenously added polyamines can reverse those damaging effects. Thus, there was strong indication that polyamines hold a pivotal role in photosynthesis, since they have been reported to be capable of simulating a photosynthetic apparatus adapted to either low or high light conditions independently of the environmental light intensity.

Thus, the present study aimed to reveal the mechanism by which polyamines can alter the structure and functioning of the photosynthetic apparatus. By exogenously supplying putrescine we established that certain changes in the levels of the polyamines putrescine and spermine bound to the photosynthetic subcomplexes are involved in the mechanism regulating the plasticity of the photosynthetic apparatus. An LHCII-associated protease activity was also found to participate in this mechanism, by being regulated by putrescine and spermine. The perspective that the polyamine changes in the photosynthetic apparatus under light adaptation can also be correlated to similar responses exerted under various environmental stresses is discussed. Thus, the unraveled mechanism is believed to be part of a pathway regulating the photosynthetic apparatus under various environmental conditions.

2. Materials and methods

2.1. Organism, growth and illumination

Cultures of the unicellular green alga *Scenedesmus obliquus*, wild type, strain D3 were grown autotrophically in liquid culture medium [33] in a temperature-controlled water bath (30 °C) in front of a set of white fluorescent lamps (L-40W, Osram, München, Germany). For the low light (LL) and high light (HL) treatments cultures were irradiated throughout the experimental period (60 h) with 50 μmoles m^{-2} s^{-1} and 200 μmoles m^{-2} s^{-1} photosynthetically active radiation (PAR) respectively. For the experiments with the polyamine Put (tetramethylenediamine dihydrochloride, Sigma-Aldrich, Germany) the compound was added to the respective cultures at 1 mM final concentration. The cultures were continuously percolated from the bottom with air thereby avoiding sedimentation.

All cultures were prepared by the inoculation of fresh medium with a stock culture (approximately 10% v/v inoculum). Care was also taken to achieve a low cell density in the starting culture (0.5 μl PCV) in order to avoid self-shading effects after growth of the cultures. By the end of the incubation period all cultures always had approximately the same cell density (0.9±0.1 μl/ml PCV).

2.2. Pigment extraction and estimation

After harvesting of the cells by centrifugation (1400×*g*, 5 min), the algal pellet was exhaustively extracted with hot methanol under safe dim green light until it was colourless. The amount of chlorophyll (Chl) was determined photometrically according to the method of Holden [34].

2.3. Polyamine analysis by HPLC

Polyamines were extracted as described by Sfichi et al. [28] and analyzed following the method of Kotzabasis et al. [35]. Briefly, for polyamine analysis cells, isolated thylakoids or photosynthetic complexes were suspended in 1 N NaOH. A volume of 0.2 ml from the hydrolysate was mixed with 36% HCl in a ratio of 1:1 (v/v) and incubated at 110 °C for 18 h. The hydrolysate was evaporated at 70–80 °C. The dried products were re-dissolved in 0.2 ml of 5% (v/v) perchloric acid. To identify and estimate the polyamines, the samples were derivatized by benzoylation, as is described by Kotzabasis and co-workers [35]. For this purpose, 1 ml of 2N NaOH and 10 μl benzoylchloride were added to 0.2 ml of the hydrolysate and the mixture vortexed for 30 s. After 20 min incubation at room temperature, 2 ml of saturated NaCl solution were added to stop the reaction. The benzoylpolyamines were extracted three times into 2–3 ml diethylether; all ether phases collected and evaporated to dryness. The remaining benzoylpolyamines were re-dissolved in 0.2 ml of 63% (v/v) methanol and 20 μl aliquots of this solution were injected into the high performance liquid chromatography (HPLC) system for the polyamine analysis, as described previously [35]. The analyses were performed with a Shimadzu Liquid Chromatography apparatus (LC-10AD) equipped with a SPD-M10A diode array detector (Shimadzu SPD-M10A) and a narrow-bore column (C18, 2.1×200 mm, 5 μm particle size Hypersyl, Hewlett-Packard, USA). To estimate directly the amount of each polyamine, the method of Kotzabasis and co-workers [35] was followed again.

The values referring to the cellular concentrations of polyamines represent total polyamines (free and bound). All the other estimations of polyamine concentrations (in thylakoid membranes and in isolated complexes) represent the bound forms.

2.4. Protein determination

Protein concentrations were determined following the method of Bradford [36], modified by Jones et al. [37].

2.5. Fluorescence induction measurements

For the fluorescence induction measurements the portable "Plant Efficiency Analyser, PEA" (Hansatech Instruments Ltd.; Kings Lynn, GB) was used. Culture samples had always a volume of 1 ml, approximately identical chlorophyll content and were collected before harvesting of the culture. Samples were adapted in darkness for 7 min before measuring. Subsequently, fluorescence curves were processed according to the JIP-method of Strasser and Strasser [38]. The method is based on the measurement of a fast fluorescence transient with a 10 μs resolution in a time span of 40 μs to 1 s. Fluorescence was measured at a 12 bit resolution and excited by 6 LEDs providing an intensity of 600 W m^{-2} of red (650 nm) light. This method allows the dynamic in vivo description of a photosynthetic sample at a given physiological state.

The equations used for the JIP-test calculations are: $ABS/RC = M_0 \cdot (1/Vj) \cdot (1/\varphi_{po})$; $RC/CSm = F_m \cdot \varphi_{po} \cdot (Vj/M_0)$; $TR_0/RC = M_0/Vj$; $DI_0/RC = (ABS/RC) - (TR_0/RC)$; $ET_0/RC = (TR_0/RC) \cdot (1 - Vj)$; $SFI = (1 - \varphi_{po}) \cdot (1 - \psi_o)$; $DF = \log\{(RC/ABS) \cdot [\varphi_{po}/(1 - \varphi_{po})] \cdot [\psi_o/(1 - \psi_o)]\}$; $\varphi_{po} = (1 - F_0/F_m)$; $M_0 = 4 \cdot (F_3 - F_0)/(F_m - F_0)$; $\psi_o = 1 - Vj$.

2.6. Determination of the packed cell volume (PCV)

The PCV of a cell suspension was determined by centrifugation at 1400×*g* for 5 min using hematocrit tubes.

2.7. Isolation of thylakoid membranes

The isolation of thylakoid membranes was performed as described by Sfichi et al. [28].

2.8. Isolation of photosynthetically active protein complexes

For the isolation of photosynthetically active protein complexes the procedure of Argyroudi-Akoyounoglou and Thomou [39] was followed. The concentration of Chl in the thylakoid sample loaded on the 5–22% continuous sucrose gradients was always adjusted to a Chl concentration of 600 μg/ml. Up to this concentration no sediment could be detected at the bottom of the gradients. In the experiment depicted in Fig. 1, the sucrose gradient had a total volume of 4.5 ml, and the total amount of chlorophyll loaded onto it was 200 μg. For the subsequent experiments all respective quantities were calculated for 10 ml volume sucrose gradients. The chlorophyll amount loaded on each gradient was thus 450 μg. Ultracentrifugation was performed at 170,000×*g* (42000 rpm, rotor SW-40, Beckman L8-80M ultracentrifuge) for 18 h at 4 °C. Fractionation of the gradients was performed using a peristaltic pump. Fractions of equal volumes (150 μl/fraction for the gradient in Fig. 1 and 450 μl/fraction for the subsequent experiments) were collected.

2.9. Detection of proteolytic activity

For the protease activity assays the procedures described in [19] were applied, with the following modifications. For the detection of the activity in gelatin-rich SDS-PAGE, the resolving gel was 10% in polyacrylamide and supplemented with 0.1% (w/v) of gelatin (Type A from porcine skin, Sigma-Aldrich, Germany). Samples of equal volumes (150 μl) of each fraction were lyophilized and diluted with 30 μl H_2O. To this mixture 0.025% (w/v) bromophenol blue was added and the samples were subsequently loaded onto the SDS-PAGE without boiling prior to electrophoresis. After electrophoresis the gels were incubated for at least 8 h at 37 °C under constant shaking in 40 mM Tris–HCl buffer, pH 8.6, with 0.2% Triton X100. Coomassie staining followed by destaining of part of the gel revealed the presence of protease activity as transparent bands on a blue background. To test for the protease activity against LHCII, the corresponding transparent bands from the unstained gel, after electrophoresis were excised and incubated with LHCII trimer. Therefore 30 μl of sample were adjusted to a concentration of 0.2 mg LHCII /ml, isolated from the sucrose gradients, and dissolved in 200 μl protease assay buffer (40 mM Tris–HCl, pH 8.6, 0.2% Triton X100). After 2 h of incubation at 37 °C, the reaction was stopped by cooling the samples to room temperature and an equal volume of solubilization buffer (4% SDS, 0.1 M Tris–HCl, pH 7.6, 8% mercaptoethanol, 10% glycerol, 0.02% bromophenol blue) was added. The remaining LHCII protein in each reaction mixture was estimated from the intensity of the staining in western blot analysis.

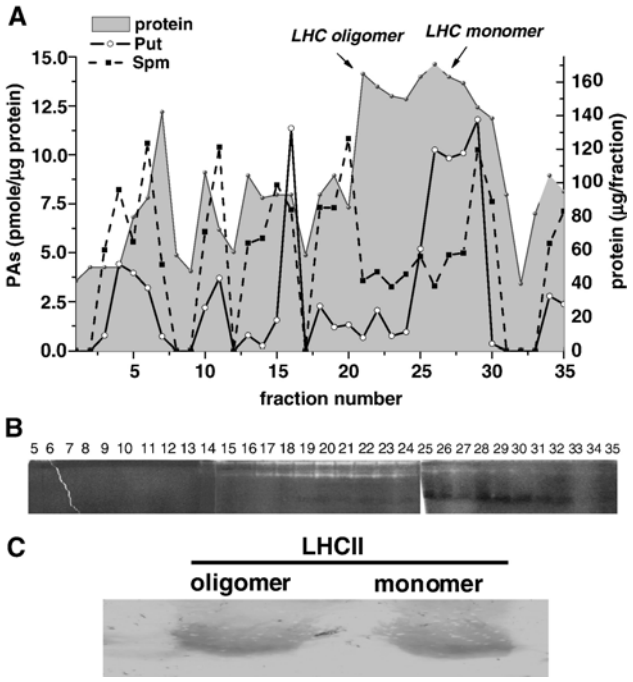

Fig. 1. (A) Changes in the protein pattern and the polyamines Put and Spm along the sucrose gradient after ultracentrifugation of the isolated thylakoids. They were prepared from a *Scenedesmus obliquus* culture grown autotrophically for 2.5 days under 100 μmol m^{-2} s^{-1} light intensity. For simplification, the polyamines of the fractions between the protein peaks are partially omitted. (B) Detection of protease activity with gelatin as substrate in SDS-PAGE. Protease activity is seen as clear bands against the dark background of the Coomassie-stained gel. The fractions (5–35) obtained from the sucrose gradient shown in (A) were assayed for protease activity. As fractions 1–5 gave no activity they were omitted. (C) Western blot immunostain of the fractions corresponding to the oligomer and the monomer LHCII, with an anti-LHCII antibody.

2.10. Western blot analyses

For the western blots the methods described in [40] were used.

3. Results

3.1. Changes in photosynthetic subcomplexes and polyamine amounts induced by the photoadaptation of the photosynthetic apparatus to different light conditions

In preliminary experiments thylakoids from *Scenedesmus* cultures grown under moderate light conditions (~100 μmol m^{-2} s^{-1}) – necessary for a sufficient yield of LHCII in the thylakoid membranes – were used to obtain subfractions of the photosynthetic apparatus by continuous sucrose gradients centrifugation (as described in Materials and methods). All fractions were analyzed for their chlorophyll, protein and polyamine contents (Fig. 1A). The results of fractionation always showed two major protein peaks, which corresponded to

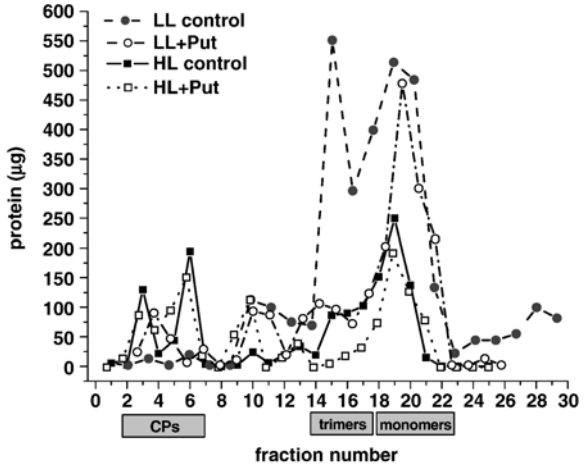

Fig. 2. Changes in the subcomplexes of the isolated thylakoids of the photosynthetic apparatus along the sucrose gradients. The bands corresponding to the LHCII oligomers and monomers and the total CPs are indicated by the gray rectangles. The fraction numbers of the four treatments were normalized along the *x*-axis to achieve a comparable visualization of the corresponding complexes.

the LHCII, as identified by western blot analysis (Fig. 1C) and Chl *a/b* ratio estimation. According to the method of Argyroudi-Akoyunoglou and Thomou [39] the two peaks were assumed to represent the oligomeric (fractions 20–24) and the monomeric (fractions 25–30) forms of LHCII, whereas fractions 5–17 were designated as the CPs of PSI with LHCI and RC II (Fig. 1A). In all other than LHCII fractions Spm was more abundant than Put. However, regarding LHCII fractions Put prevailed compared to Spm in the monomeric form of LHCII, whereas its concentration was lower than Spm in the oligomeric complexes (Fig. 1A). Thus it should be possible to modulate the endogenous polyamine levels by adding Put. This should result in drastical alterations of the Put/Spm ratio.

Therefore, a stock culture adapted to moderate light conditions (100 µmol m^{-2} s^{-1}) was used to inoculate a series of *Scenedesmus* cultures which were then newly adapted for 60 h either in low (50 µmol m^{-2} s^{-1}) or in high (200 µmol m^{-2} s^{-1}) light conditions. Thylakoid subfractions were obtained as described above. The protein content of the fractions along the gradients is shown in Fig. 2, and summarized in Table 1. Indeed, the different light adaptations of the cultures resulted in great differences, mainly in the amount of the overall LHCII, but also in its subcomplexes, as well as in the total CPs, as expected (Table 1): in high light (HL) the total amount of LHCII was lower (~65%) than in low light (LL). Compared to LL, the HL treatment showed a decrease in both oligomer (~80%) and monomer (~60%) LHCII subcomplexes. Great differences were also observed with the CPa (the RC of PSII) and CPIa (the RC of PSI plus LHCI) complexes: in LL the sum of CPs (Figs. 1 and 2A, fractions 1–6) was 40 µg, while in HL (Fig. 2, fractions 1–6) it was 417 µg — and thus about 10 times higher (Table 1).

Simultaneously, related to whole cells the amount of Put remained more or less unchanged under LL and HL conditions, while Spm had increased to double in the case of HL conditions (Table 2). Referred to the thylakoid basis, Put was reduced by approximately 50%, while Spm remained almost unchanged (Table 2). Analysis of polyamine levels in the oligomeric and monomeric forms of LHCII revealed that a higher light intensity induced an increase in the concentrations of both Put and Spm bound to LHCII, compared to LL intensity (Fig. 3, Table 3). However, the increase in Put was higher in the oligomers, while Spm had increased more in the monomers. In HL LHCII-oligomers, Put concentration was upregulated approximately by 540% compared to LL and Spm was found to be increased by 560%. Vice versa, in HL the Put of the monomers was increased by 370% compared to LL, while at the same time Spm had increased by 640%. In contrast, the CPs lost a considerable amount of their bound polyamines: 38% in Put and 65% in Spm. Conclusively the significant differences between HL and LL adaptation are due to concentration changes in Put and Spm in the LHCII subcomplexes (Fig. 3, Table 3).

3.2. Exogenously supplied polyamines induce the reorganization of the photosynthetic apparatus

In a parallel experiment, besides a control, LL and HL cultures were supplied with 1 mM Put at the time of inoculation. Determination of the polyamines in both, whole cells and thylakoid preparations, verified that Put was successfully incorporated into the plastids and also was bound to the thylakoid membranes (Table 2). However, while external Put supply under both LL and HL conditions, referring to whole cells resulted in a great induction of Spm, no increment of this polyamine was found in the thylakoid membranes meaning that the Spm levels bound to the thylakoid membranes showed only minor changes (Table 2).

Furthermore, the addition of exogenous Put, resulted in a decrease of the total LHCII content and an increase in total CPs

Table 1
Amounts of total protein (in µg) in the fractions of the sucrose gradient, in the LHCII, its subcomplex fractions, and the CPs, as well as the ratio of LHCII/CPs

	LL						HL					
	Total protein	LHCII oligomer	LHCII monomer	Total LHCII	Total CPs	LHCII/CPs	Total protein	LHCII oligomer	LHCII monomer	Total LHCII	Total CPs	LHCII/CPs
Control	3116	915	1529	2445	40	61.0	1352	196	640	836	396	2.11
+Put	1996	354	1001	1355	197	6.8	1194	59	396	454	433	1.05
% change	−36%	−60%	−34%	−45%	+492%	−89%	−12%	−70%	−38%	−45%	+9.3%	−50%

The values for the four treatments (Control LL, LL+Put, Control HL, HL+Put) were obtained by summing up the protein amounts measured in the fractions corresponding to each complex (see also Fig. 3).

Table 2
Changes in the concentration of Put and Spm on a cellular level, expressed in pmol per μl PCV and in isolated thylakoid membranes, expressed in pmol per μg of total protein at two different light intensities: low light (LL, 50 μmol m^{-2} s^{-1}) and high light (HL, 200 μmol m^{-2} s^{-1}), with or without the addition of 1 mM Put

	Cells		Thylakoids	
	PUT (pmol/μl PCV)	SPM (pmol/μl PCV)	PUT (pmol/μg protein)	SPM (pmol/μg protein)
LL	1380±321	405±67	85.7±3	33.8±4
LL+Put	21700±2466	720±245	757.6±81	39.7±2.3
HL	1314±258	750±69	47.6±0.66	32.4±6
HL+Put	23150±6505	947±142	206.7±25	29.4±4.2

The *Scenedesmus obliquus* cultures were grown autotrophically for 2.5 days.

Table 3
Polyamine concentrations in isolated LHCII subcomplexes (oligomers and monomers) and in total CPs, after ultracentrifugation on sucrose gradients of the isolated thylakoids

	Polyamines (pmol/μg protein)		Polyamines (pmol/μg protein)	
	Put	Spm	Put	Spm
	LL		HL	
LHCII oligomer	2.80	8.28	15.06	46.27
LHCII monomer	3.80	4.87	14.16	31.11
CPs	38.20	80.30	16.14	27.60
	LL+Put		HL+Put	
LHCII oligomer	751.40	43.00	282.10	441.2
LHCII monomer	326.00	13.60	60.50	59.20
CPs	830.50	43.70	26.70	61.20

The *Scenedesmus obliquus* cultures were grown autotrophically for 2.5 days.

(RCs+LHCI), both in LL and in HL (Fig. 2, Table 1). On the one hand, the addition of Put under LL conditions resulted in approximately 45% reduction of LHCII complexes and at the same time in an 490% induction of the CPs, compared to the corresponding control culture. On the other hand, in HL, exogenously added Put resulted in 45% reduction of the LHCII but only a 9% induction of the CPs could be observed (Table 2). Related to the LHCII subcomplexes, the decrease in oligomers was greater than that of the monomers. Under LL conditions, the Put-supplemented cultures showed approximately 60% reduction in oligomers and 34% reduction in monomers. In HL conditions, the respective changes initiated by Put addition were

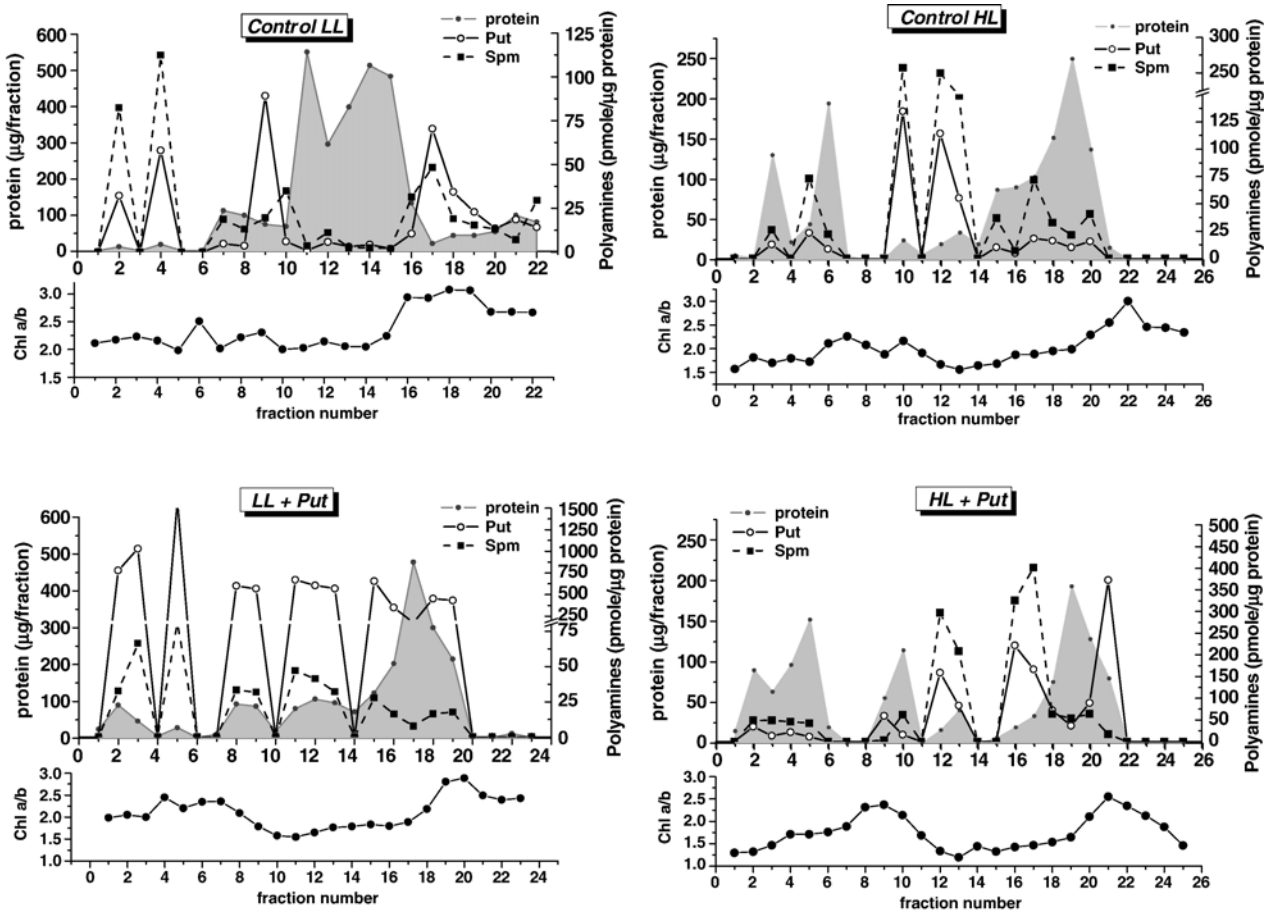

Fig. 3. Changes in the protein pattern and the polyamines Put and Spm of the isolated thylakoids along the sucrose gradients after ultracentrifugation. The thylakoids were prepared from *Scenedesmus obliquus* cultures grown autotrophically for 2.5 days under LL (50 μmol m^{-2} s^{-1}) or HL (200 μmol m^{-2} s^{-1}) conditions, either without (control) or supplemented with 1 mM Put. The Chl *a*/*b* ratio for all values is also depicted. For simplification, the polyamine concentrations of the fractions between the protein peaks are partially omitted. Thylakoids loaded on each gradient contained always 450 μg of Chl.

similar with a 70% reduction in oligomers at 38% in monomers (Table 1).

After the addition of exogenous Put, either in HL or in LL, both types of LHCII subcomplexes were found to be loaded with excess Put compared to the controls (Fig. 3, Table 3). Again, the oligomers were more loaded with Put than the monomers. The Spm concentration was also increased in all cases, but similarly to Put, the increase was greater in the oligomers than in the monomers. Referring to the light conditions, the Put overload in both LHCII fractions was lower under HL conditions, than in LL (Table 3). This fact is also reflected in the concentration of this polyamine in the respective thylakoid membrane preparations: LL+Put treatment resulted in a total Put concentration of 757 pmol/μg thylakoid protein, whereas the respective value in the case of HL was 206 pmol/μg protein (Table 2).

The examination of the ratio of Spm per Put (Spm/Put), in all gradient fractions obtained from the four different culture treatments discussed above, revealed an interesting feature: at LL (Control-LL) conditions the ratio showed a great difference in the LHCII subcomplexes, namely very high values in the oligomers and very low values in the monomers. Under HL conditions (Control-HL) the difference between the two types of subcomplexes was somewhat smoothened i.e. a lowered ratio in the oligomers and an elevated ratio in the monomers (Fig. 4). As already stated, Put addition in LL conditions resulted in a great increase of the Put concentration in all gradient fractions, with conclusively very low absolute Spm/Put ratios. However, the same trend as under HL conditions became visible: an almost equal Spm/Put ratio in oligomers and monomers. Finally, in the case of HL+Put treatment, in both LHCII subcomplexes the absolute value of the ratio was lowered, whereas the difference between them remained almost unchanged, comparable to the Control-HL and the LL+Put treatments.

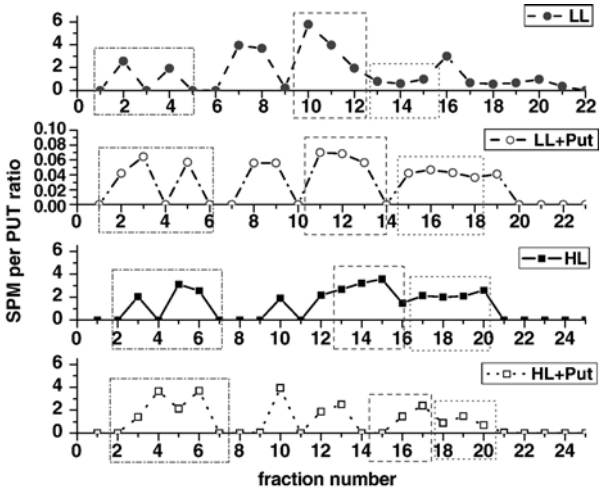

Fig. 4. Calculated values of Spm/Put ratios along all gradient fractions of the LL and HL treatments, with or without Put addition to the culture. The dashed rectangle indicates the fractions corresponding to the LHCII oligomers, the dotted one indicates the fractions corresponding to the LHCII monomers, and the dash-dotted one indicates the fractions corresponding to the CPs, based on protein bands and Chl *a*/*b* values shown in Fig. 3. The original values of Put and Spm concentrations are also depicted in Fig. 3.

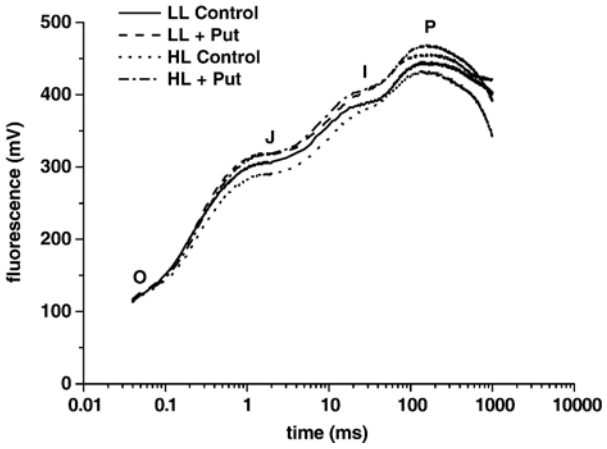

Fig. 5. Fluorescence induction curves recorded from the four cultures: LL Control; LL+Put; HL Control; HL+Put. The O–J–I–P steps of the fluorescence transients are indicated.

3.3. Study of the molecular structure and functioning of the photosynthetic apparatus by chlorophyll fluorescence induction measurements and JIP-test analysis

Also, chlorophyll fluorescence measurements were used to screen the physiological and functional state of the photosynthetic apparatus in all cultures (Fig. 5). The respective results of the JIP-test processed data (including the explanation of the JIP-test abbreviations) are summarized in Table 4. The data show that addition of Put to the cells initiates changes in the photosynthetic apparatus resembling those induced by higher light intensity. Referring to the LL control, the LL+Put treated culture resembled a culture adapted to higher light intensity, though the values were lower than for the HL control culture. Likewise, the HL+Put culture exhibited an overall picture of a culture adapted to even higher light intensity than the HL control culture itself (Table 4). The ABS/RC, a parameter standing for the functional size of the LHCII antenna

Table 4
Presentation of selected JIP-test calculated parameters from chlorophyll fluorescence data measured from LL, LL+Put, HL and HL+Put cultures

JIP-test parameter		LL	LL+Put	HL	HL+Put
Photosynthetic efficiency	F_v/F_m	0.723	0.722	0.718	0.735
Functional size of LHCII	ABS/RC	3.373	3.313	3.183	3.001
Active RC density per cross section at F_m	RC/CSm	1.313	1.376	1.357	1.563
Trapping efficiency of PSII per RC	TRo/RC	2.438	2.394	2.286	2.206
Electron transport per active RC	ETo/RC	1.035	1.009	1.053	0.963
Dissipation energy per active PSII RC	DIo/RC	0.935	0.919	0.897	0.795
Structure–Function Index	SFI	0.910	0.919	1.039	1.069
Driving force of photosynthesis	DF	1.741	1.744	1.922	1.968

The original fluorescence curves have been normalized for minor chlorophyll concentration differences among the samples. All data are expressed in arbitrary units.

per reaction center, is decreased in the order LL>LL+Put>HL>HL+Put which is to some extent also true for the trapping efficiency per active reaction center (TRo/RC) and the energy dissipated per reaction center (DIo/RC). Similarly, the density of the active reaction centers (RC/CSm) increased in the same order. However, the overall driving force (DF) of photosynthesis seems to be unaffected by Put and based only on the different light intensities. The obtained values for LL and LL+Put, 1,741 and 1,744 are very similar, as are those for HL and HL+Put 1,922 and 1,968, but a considerable difference exists between the two pairs. Concerning the SFI factor (Structure–Function Index), again little difference is observed within the two pairs however, it is considerable between the two pairs of SFI values (Table 4).

Remarkably, none of the cultures showed however any indication of photoinhibition or stress, as revealed by the shape of the fluorescence curves (Fig. 5) and the JIP-test processing. Also, the photosynthetic efficiency F_v/F_m did not vary significantly within the four cultures (Table 4).

As shown, the physicochemical analysis fully confirmed the above described biochemical data. Similar preliminary experiments, using instead of Put its inhibitor 1,4-diamino-2-butanone, yielded opposite results: an increase in both the monomers and the oligomers of LHCII and a decrease in the CPs, even when relatively LL conditions were applied (data not shown). This fact was indicated in a previous publication too [27].

3.4. Autoproteolytic activity of LHCII — the polyamine impact

The above described data fundamentally establish the important role of polyamines and especially of the diamine Put in the structural and functional flexibility during the light adaptation of the photosynthetic apparatus. This leads to the question whether this drastic effect of polyamines on the aggregation/disaggregation state of LHCII is exerted through an autoproteolytic activity of the complex which has been reported to exist in higher plants [40]. Therefore, fractions of the photosynthetic apparatus of *S. obliquus* were prepared by sucrose gradient ultracentrifugation, and tested for proteolytic activity on gelatin containing polyacrylamide gels to detect the postulated protease (Fig. 1B). Proteolytic activity was found from fraction 17 through fraction 24, with a maximum in fraction 20. The oligomer of LHCII ran in fractions 21 to 24. No activity was detected in the fractions 25–31, attributed to the monomers (Fig. 1B). Moreover, this protease activity was found to comprise of an upper and a lower band, both of high molecular masses and thus remaining at the beginning of the resolving gel (10% polyacrylamide).

To find out whether polyamines directly affect the protease activity, isolated oligomer preparations of LHCII were employed. Therefore the polyamines putrescine, spermidine and spermine were added to the LHCII oligomer preparation at final concentrations ranging from 0.01 mM to 2.0 mM and after a 2 h incubation at 37 °C the autoproteolytic activity indirectly estimated by measuring the remaining oligomer LHCII protein in the reaction mixtures (Fig. 6). The results showed that small Put concentration of 0.01–0.5 mM in the reaction mixture negatively affected the autoproteolytic activity, since they restored up to 90% of the remaining oligomeric LHCII complex in the mixture, compared to the control with only oligomer LHCII. At higher polyamine concentrations (0.5–2 mM), however, the inhibitory effect of Put on the autoproteolytic activity declined by lowering the amount of remaining oligomeric LHCII in the mixture. Also Spm showed a similar reaction at low concentrations, although its negative effect was approximately 30% lower than in that of Put. It increased only a little its inhibitory effect on the LHCII autoproteolytic activity at concentrations of 1 to 2 mM. The triamine spermidine (Spd) had more or less no effect on the protease activity (Fig. 6A). Also with gelatine as substrate, low concentrations of Put resulted in a lowering of the autoproteolytic activity of the LHCII, whereas Put concentrations >1 mM directly increased the activity (Fig. 6B). The gain in protease activity even exceeded the control without Put by far when it was applied at concentrations of 2.5 mM or higher (Fig. 6B).

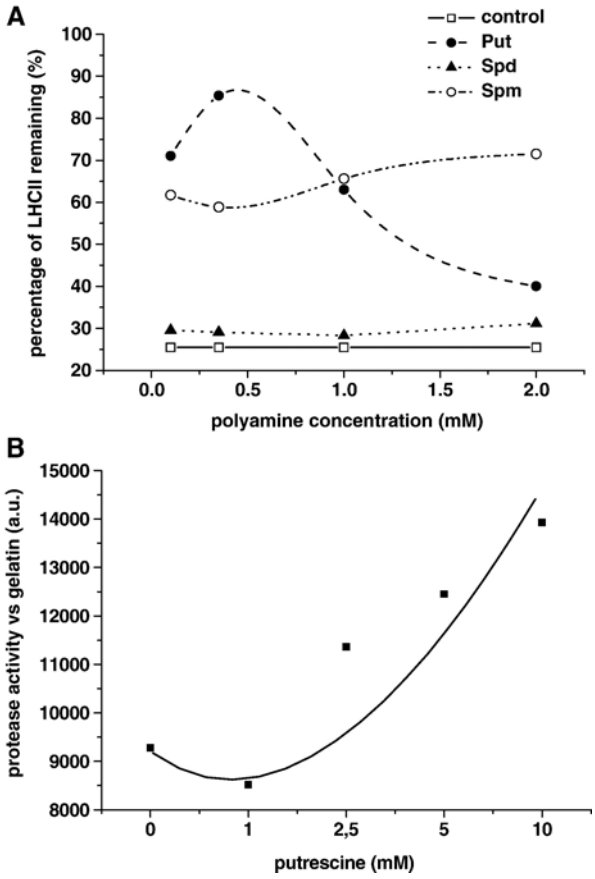

Fig. 6. *In vitro* effect of externally added polyamines to the proteolytic activity of isolated trimer LHCII. (A) Put, Spd, and Spm were added to the reaction mixture, containing isolated LHCII-oligomers (0.2 mg/ml), at concentrations ranging from 0.1 to 2 mM. Autoproteolytic activity is expressed as % of the remaining LHCII detected by immunoblot analysis of the reaction mixture after 2 h incubation at 37 °C. The control represents the activity of the reaction mixture without any polyamine addition. (B) Put, in concentrations ranging from 0 to 10 mM, was added to the reaction mixture containing isolated LHCII-oligomers (0.2 mg/ml). The proteolytic activity, expressed in arbitrary units, was determined with gelatin as substrate in the gel. For details see Materials and methods.

4. Discussion

Our previous studies provided many indications that the polyamines Put and Spm induce changes in the photosynthetic apparatus which simulate an adaptation to either high or low light intensity [27]. It is also generally accepted that in many cases of environmental stress stimuli the photosynthetic apparatus adopts certain adjustments that resemble an adaptation to either low or high light conditions [28–32], this explaining the protective role of exogenously supplied polyamines. However, the mechanism by which polyamines confer this action is still unknown. The following discussion of our results will elucidate, at least in part, this obviously more general mechanism.

4.1. Polyamines adjust the structure and bioenergetics of the photosynthetic apparatus

Our data clearly show that the externally supplied Put can enter the cells, raising its intracellular level under both HL and LL conditions (Table 2), without causing any toxic effects or photoinhibition [41] (Fig. 5). Also, Put entered the chloroplast and was bound to thylakoids (Table 2) albeit with a differential effectiveness between the LL (757 pmol/μg protein) and HL (206 pmol/μg protein) treatments.

In the alga *S. obliquus* the bound polyamines were found to be associated with both the oligomers and the monomers of LHCII, as well as with the CPs thus resembling higher plants [23]. However, the distribution does not reflect a constant pattern; in LHCII subcomplexes Put and Spm levels fluctuated depending on the light adaptational status of the photosynthetic apparatus.

It is astonishing that the mere application of Put to the cells is capable to cause a total reorganization of the photosynthetic apparatus as if the cultures were transferred to a higher than the actual light intensity [42–46]. This was certified both, by the biochemical analysis of photosynthetic subcomplexes and by its physicochemical analysis (decrement of ABS/RC, TRo/RC, and DIo/RC and increment of RC/CSm). Indeed, LL+Put- as well as HL+Put-treated cells developed a photosynthetic apparatus with a significantly lower amount of both, oligomeric and monomeric LHCII and a higher amount of CPs, compared to the controls. The number of CPs was strongly increased under LL+Put conditions whereas the respective increase in HL+Put was low. Comparison of HL to the LL controls revealed that the big differences in bound polyamines were localized in the LHCII subcomplexes (approximately 370–560% increase in bound Put and Spm), rather than in the CPs (38–65% increase in bound Put and Spm, Table 3). It thus seems reasonable that the effect of the overall reorganization of the photosynthetic apparatus by Put is mainly due to the changes provoked by the amounts of polyamines bound to the LHCII subcomplexes. Noteworthy, Put addition, either under LL or HL conditions, resulted in about the same percentage of reduction of the total LHCII and its subcomplexes.

In general, chlorophyll fluorescence analysis points out to an improved capacity for photochemical utilization of the energy flow after Put treatment. However, it seems that there is a discrepancy between the capability for light utilization by the structural status of the photosynthetic apparatus conditioned by Put and the actual light intensity perceived: despite the overall reorganization of the photosynthetic apparatus towards an adaptation to higher light by the addition of Put, the incident photon flow rate remains constant. In other words, due to the impact of Put the photosynthetic apparatus has been enabled to perceive more of the incident light. Hence, the actual light intensity perceived by the cells does not correspond to a "new" structural reorganization of the photosynthetic apparatus, which has been adapted to confront a higher fluence rate. This is indicated by the overall driving force of photosynthesis (DF), as well as the structure–function index (SFI), which show very similar values for the treatments with and without Put at the same light intensity (Table 4). This explanation is also supported by the data on the rate of electron transport per reaction center (ETo/RC) which was found to be reduced instead of being increased: although there are more reaction centers to supply with electrons, however the "limited" light conditions reduce the electron flow per active reaction center. Altogether, this explanation fits also with the protective role of Put against the impact of many environmental stresses on the photosynthetic apparatus. Indeed, in many cases of environmental stress stimuli the photosynthetic apparatus has been proved to adopt certain adjustments that simulate a low light adaptation of the photosynthetic apparatus [28,29,31,32]. Thus, the actual incident light is perceived as a high light stress and induces an excitation pressure. Put addition can reverse this phenomenon: it enhances the tolerance against the stressor by enabling the reorganization of the photosynthetic apparatus to a higher light adapted one. In full agreement to the above mentioned, previous contributions showed that a low Spm/Put ratio enhances the tolerance of plants against high UV-B radiation [28,29], cold stress [32] and increased tropospheric ozone [31].

4.2. The polyamine-induced reorganization of the photosynthetic apparatus may originate from a direct Put effect on the autoproteolytic activity of the LHCII

One of the primary mechanisms that probably participate in the phenomena described so far must be the proteolysis of LHCII oligomers, since invariably these subcomplexes are being downregulated by Put. LHCII oligomers have been found to possess autoproteolytic activity. This activity is due to a membrane-bound protease of high molecular mass (140 kDa), which is a member of the cysteine family of proteases. It is closely associated with LHCII trimers in vivo, and additionally is capable of degrading the LHCII monomers, but also exhibits proteolytic activity against the D1 and D2 proteins of the PSII reaction center [40].

Our attempts to detect this proteolytic activity in *S. obliquus* too, were successful (Fig. 1B) and moreover, this protease was found to have very similar characteristics as the one reported for higher plants (for comparisons see also [40]). Interesting is the fact that from our results the proteolytic activity (Fig 1B, fractions 17–24) is not only located exactly upon the LHCII oligomers (Fig. 1A, fractions 21–24), but is here extended to a

protein complex that is always present beside LHCII oligomers (Fig. 1A, fractions 17–20). The reason for this discrepancy may be that in the case of the higher plants the amount of total chlorophyll was plotted as the pattern of the photosynthetic complexes along the sucrose gradient, whereby minor protein complexes with lower Chl content, like the one discussed here, would most probably be ignored.

Further in vitro experiments on the proteolytic activity with externally supplied polyamines and isolated LHCII, revealed that high Spm/Put ratios might also augment the inhibitory effect on the protease activity, as it was shown for the single polyamines (Fig. 6). Vice versa, a low Spm/Put ratio could reduce the inhibition exerted by either of the two polyamines and even enhance the activity. There are at least two other reports revealing an inhibitory effect of Spm on protease activities [47,48] this proposing a common mechanism for different classes of organisms. All this matches very well with the effect of the Spm/Put ratio on changes in the LHCII complexes and supports our hypothesis.

Interestingly, the same band migrating just below LHCII oligomers complex, and containing the proteolytic activity shows transglutaminase activity, as recently reported by Della Mea et al. [49]. This band is recognized to comprise an LHCII–CP29–CP26 supercomplex [50,51]. Thus, the autoproteolytic activity in the LHCII oligomers, which is influenced by Put and Spm, is co-localized with that transglutaminase, which is responsible for the light-dependent binding of these polyamines to the LHCII complex. It is noteworthy that all known mammalian forms of TGases known today belong to the papain-like superfamily of cysteine proteases [22,52], thus strikingly sharing some characteristics with the detected protease.

4.3. Proposed mechanism

A possible explanation of our current results could be that molecular perturbations act on the photosynthetic protein complexes produced by the polycationic nature of the polyamine molecules. Indeed, both the strength of the positive charge and the backbone length of Spm and Put, 1.46 nm and 0.65 nm respectively, differ significantly, while potentially they antagonize for the same glutamate residues on polypeptide molecules. Thus, their effects upon binding to polypeptides are expected to be quite different. An earlier study reported that Spm, and to a lesser extent Spd too, can cause a significant decrease in the percentage of a-helices with concomitant increase in the β-sheet domains in isolated PSII-enriched fractions [47,53]. Unfortunately, no similar data exist on Put effects. Therefore, the modification of the secondary structure of LHCII and CPs by any of the polyamines could drastically affect their conformation and either stabilize them or expose them to protease activity. Such a modification could originate in both, LHCII subcomplexes and the CPs separately, or primarily originate in one of them (more possibly on the LHCII oligomers) and by a cascade mechanism affect the other complexes, too. Further studies are needed to prove this hypothesis.

A recent publication on the adaptive, irradiance-dependent changes in the different subpopulations of LHCII trimers reported that the most acidic complexes (identified by ndIEF) are those that are gradually decreased at increasing light intensities [54]. The remaining less acidic ones could be those that had been loaded to a greater extent with polyamines. Among the polypeptides Lhcb1–3 comprising the different LHCII subpopulations, there are significant differences in abundance, both, spatially and temporally due to their specific roles in light acclimation [54–56]. The observed difference in the Chl a/*b* ratio among the three polypeptides was hypothetically attributed to a differential accumulation of Lhcb1–3 depending on the light and/or temperature conditions [50,51,55]. Our results clearly present a change in the Chl a/*b* ratio, both, in the oligomers and the monomers of LHCII, with values of 2.02 in the trimers and 2.04 in monomers in LL. The values are lowered to 1.55 and 1.88, respectively, in LL + Put. In HL ratios of 1.68 and 1.95 were obtained, lowered to 1.32 and 1.64 with HL + Put. These data clearly demonstrate a differential polypeptide and pigment composition of LHCII during both natural photoacclimation and Put-induced acclimation. This raises the still open question whether the mechanism of polyamine-mediated LHCII modification distinguishes between the different Lhcb polypeptides.

5. Conclusions

Polyamines and specifically Put are able to induce the total reorganization of the photosynthetic apparatus simulating high light photoadaptation, independent of the environmental light conditions. Defined changes in the Spm/Put ratios bound to photosynthetic subcomplexes and not of the polyamine (Put and Spm) amounts per se, stimulate the structural and functional reorganization of the photosynthetic apparatus. A mechanism is proposed, explaining the high capability of exogenously added endogenously increased polyamines to alter the adaptational status of the photosynthetic apparatus and to confer protection against environmental stimuli. The mechanism is based on the direct effects of polyamines on the autoproteolytic activity of LHCII. It plays a central, regulatory role and is therefore of great significance.

Acknowledgements

We heartily thank Dr. J.H. Argyroudi-Akoyunoglou from the Demokritos Research Institute of Athens for scientific contribution and helpful advice and Dr. Dieter Dörnemann for his invaluable contribution in editing the manuscript. We also thank Dr. M. Arkas for methodological support and G. Sofou for technical assistance.

This work was supported by the Hellenic General Secretariat for Research and Technology.

References

[1] K. Kotzabasis, A role for chloroplast-associated polyamines? Bot. Acta 109 (1996) 5–7.
[2] A.N. Webber, N.R. Baker, Control of thylakoid membrane development and assembly, Oxygenic photosynthesis: the light reactions, Kluwer Acad. Publ, The Nederlends, 1996, pp. 35–41.

[3] R.G. Walters, J.J.M. Rogers, F. Shephard, P. Horton, Acclimation of *Arabidopsis thaliana* to the light environment: the role of photoreceptors, Planta 209 (1999) 517–527.
[4] T. Pfannschmidt, Chloroplast redox signals: how photosynthesis controls its own genes, Trends Plant Sci. 8 (2003) 33–41.
[5] V. Fey, R. Wagner, K. Brautigam, M. Wirtz, R. Hell, A. Dietzmann, D. Leister, R. Oelmuller, T. Pfannschmidt, Retrograde plastid signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*, J. Biol. Chem. 280 (2005) 5318–5328.
[6] R.G. Walters, Towards an understanding of photosynthetic acclimation, J. Exp. Bot. 56 (2005) 435–447.
[7] P. Fleischhacker, H. Senger, Adaptation of photosynthetic apparatus of *Scenedesmus obliquus* to strong and weak light conditions, Physiol. Plant. 43 (1978) 43–51.
[8] H. Senger, B. Bauer, The influence of light quality on adaptation and function of the photosynthetic apparatus, Photochem. Photobiol. 45 (1987) 939–946.
[9] M.R. Webb, A. Melis, Chloroplast response in *Dunaliella salina* to irradiance stress, Plant Physiol. 107 (1995) 885–893.
[10] C. Wilhelm, P. Krämer, A. Wild, Effect of different light qualities on the ultrastucture, thylakoid membrane composition and assimilation metabolism of *Chlorella fusca*, Physiol. Plant. 64 (1985) 359–364.
[11] H. Dau, Short-term adaptation of plants to changing light intensities and its relation to photosystem II photochemistry and fluorescence emission, J. Photochem. Photobiol., B Biol. 26 (1994) 3–27.
[12] P. Horton, A.V. Ruban, A.J. Young, Regulation of the structure and function of the light harvesting complexes of photosystem II by the xanthophyll cycle, in: A.J. Yang, H.A. Frank, G. Britton, R.J. Cogdell (Eds.), The Photochemistry of Carotenoids, Chapter 15, Kluwer Acad. Publ, The Netherlands, 1999, pp. 271–291.
[13] D.R. Ort, When there is too much light, Plant Physiol. 125 (2001) 29–32.
[14] X.-P. Li, A.M. Gilmore, S. Caffari, R. Bassi, T. Golan, D. Kramer, K. Niyogi, Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein, J. Biol. Chem. 279 (2004) 22866–22874.
[15] A.A Pascal, Z. Liu, K. Broess, B. van Oort, H. van Amerongen, C. Wang, P. Horton, B. Robert, W. Chang, A. Ruban, Molecular basis of photoprotection and control of photosynthetic light-harvesting, Nature 436 (2005) 134–137.
[16] W. Kühlbrandt, D.N. Wang, Y. Fujiyoshi, Atomic model of plant light harvesting complex, Nature 350 (1994) 614–621.
[17] E.J. Boekema, H. van Roon, F. Calkoen, R. Bassi, J.P. Dekker, Multiple types of association of photosystem II and its light-harvesting antenna in partially solubilized photosystem II membranes, Biochemistry 38 (1999) 2233–2239.
[18] G. Jackowski, K. Kacprzak, S. Jansson, Identification of Lhcb1/Lhcb2/Lhcb3 heterotrimers of the main light-harvesting chlorophyll a/b-protein complex of photosystem II (LHCII), Biochim. Biophys. Acta 1504 (2001) 340–345.
[19] R. Anastassiou, J.H. Argyroudi-Akoyunoglou, Thylakoid bound proteolytic activity against LHC-II apoprotein in bean, Photosynth. Res. 43 (1995) 241–250.
[20] D.-H. Yang, M. Lindahl, Z. Adam, B. Andersson, A thylakoid-bound proteolytic activity involved in acclimative degradation of LHCII, in: P. Mathias (Ed.), Photosynthesis: from Light to Biosphere, vol. IV, Kluwer Acad Publ, The Netherlands, 1995, pp. 453–456.
[21] R. Besford, C. Richardson, J. Campos, A.F. Tiburcio, Effect of polyamines on stabilization complexes in thylakoid membranes of osmotically stressed oat leaves, Planta 189 (1993) 201–206.
[22] S. Del Duca, L. Doldini, M. Della Mea, P. Munoz de Rueda, D. Serafini-Fracassini, Factors affecting transglutaminase activity catalysing polyamine conjugation to endogenous substrates in the entire chloroplast, Plant Physiol. Biochem. 38 (2000) 429–439.
[23] K. Kotzabasis, C. Fotinou, K.A. Roubelakis-Angelakis, D. Ghanotakis, Polyamines in the photosynthetic apparatus. Photosystem II highly resolved subcomplexes are enriched in spermine, Photosynth. Res. 38 (1993) 83–88.
[24] S. Del Duca, V. Tidu, R. Bassi, C. Esposito, D. Serafini-Fracassini, Identification of chlorophyll-a/b proteins as substrates of transglutaminase activity in isolated chloroplasts of *Helianthus tuberosus* (L.), Planta 193 (1994) 283–289.
[25] K. Kotzabasis, E. Navakoudis, G. Tsolakis, H. Senger, D. Dörnemann, Characterization of the photoreceptor(s) responsible for the regulation of the intracellular polyamine level and the putative participation of heterotrimeric G-proteins in the signal transduction chain, J. Photochem. Photobiol., B Biol. 50 (1999) 38–44.
[26] A. Beigbeder, M. Vavadakis, E. Navakoudis, K. Kotzabasis, Influence of polyamine inhibitors on light-independent and light-dependent chlorophyll biosynthesis and on the photosynthetic rate, J. Photochem. Photobiol., B Biol. 28 (1995) 235–242.
[27] K. Kotzabasis, B. Strasser, E. Navakoudis, H. Senger, D. Dörnemann, The regulatory role of polyamines in structure and functioning of the photosynthetic apparatus during photoadaptation, J. Photochem. Photobiol., B Biol. 50 (1999) 45–52.
[28] L. Sfichi, N. Ioannidis, K. Kotzabasis, Thylakoid-associated polyamines adjust the UV-B sensitivity of the photosynthetic apparatus by means of light-harvesting complex II changes, Photochem. Photobiol. 80 (2004) 499–506.
[29] C. Lütz, E. Navakoudis, H.K. Seidlitz, K. Kotzabasis, Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UV-B protection, Biochim. Biophys. Acta 1710 (2005) 24–33.
[30] K. Logothetis, S. Dakanali, N. Ioannidis, K. Kotzabasis, The impact of high CO2 concentration on the structure and function of the photosynthetic apparatus and the role of polyamines, J. Plant Physiol. 161 (2004) 715–724.
[31] E. Navakoudis, C. Lütz, C. Langebartels, U. Lütz-Meindl, K. Kotzabasis, Ozone impact on the photosynthetic apparatus and the protective role of polyamines, Biochim. Biophys. Acta 1621 (2003) 160–169.
[32] M. Sfakianaki, L. Sfichi, K. Kotzabasis, The involvement of LHCII-associated polyamines in the response of the photosynthetic apparatus to low temperature, J. Photochem. Photobiol., B Biol. 84 (2006) 181–188.
[33] N.I. Bishop, H. Senger, Preparations and photosynthetic properties of synchronous cultures of Scenedesmus, in: A. San Pietro (Ed.), Methods in Enzymology, vol. 23, part A, Acad. Press, New York, 1971, pp. 53–66.
[34] M. Holden, Chlorophylls, in: T.W. Goodwin (Ed.), Chemistry and Biochemistry of Plant Pigments, Academic Press, London, 1965, pp. 461–488.
[35] K. Kotzabasis, M.D. Christakis-Hampsas, K.A. Roubelakis-Angelakis, A narrow-bore HPLC method for the identification and quantitation of free, conjugated and bound polyamines, Anal. Biochem. 214 (1993) 484–489.
[36] M.M. Bradford, A rapid and sensitive method for the estimation of microgram quantities of protein utilising the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[37] C. Jones, D. Hare, S. Compton, Measuring plant protein with Bradford assay. Evaluation and standard method, J. Chem. Ecol. 15 (1989) 979–992.
[38] B.J. Strasser, R.J. Strasser, Measuring fast fluorescence transients to address environmental questions: the JIP-test, in: P. Mathias (Ed.), Photosynthesis: from Light to Biosphere, vol. V, Kluwer, Dordrecht, 1995, pp. 977–980.
[39] J.H. Argyroudi-Akoyunoglou, H. Thomou, Separation of thylakoid pigment–protein complexes by SDS-sucrose density gradient centrifugation, FEBS Lett. 135 (1981) 177–181.
[40] J.H. Georgakopoulos, A. Sokolenko, M. Arkas, G. Sofou, R.G. Hermann, J.H. Argyroudi-Akoyounoglou, Proteolytic activity against the light-harvesting complex and the D1/D2 core proteins of Photosystem II in close association to the light-harvesting complex II trimer, Biochim. Biophys. Acta 1556 (2002) 53–64.
[41] S. Govindachary, N.G. Bukhov, D. Joly, R. Carpentier, Photosystem II inhibition by moderate light under low temperature in intact leaves of chilling-sensitive and -tolerant plants, Physiol. Plant 121 (2004) 322–333.
[42] U.K. Larsson, J.M. Anderson, B. Anderson, Variations in the relative content of the peripheral and inner light-harvesting chlorophyll *a/b*-protein complex (LHCII) subpopulations during thylakoid light adaptation and development, Biochim. Biophys. Acta 894 (1987) 69–75.

[43] T.Y. Leong, J.M. Anderson, Adaptation of the thylakoid membranes of pea chloroplast to light intensities: I. Study on the distribution of chlorophyll-protein complexes, Photosynth. Res. 5 (1987) 117–128.
[44] J.M. Anderson, W.S. Chow, D.J. Goodchild, Thylakoid membrane organization in sun/shade acclimation, Aust. J. Plant Physiol. 15 (1988) 11–26.
[45] W.S. Chow, E.-H. Kim, P. Horton, J.M. Anderson, Granal stacking of thylakoid membranes in higher plant chloroplasts: the physicochemical forces at work and the functional consequences that ensue, Photochem. Photobiol. Sci. 4 (2005) 1081–1090.
[46] I.A. Papadakis, K. Kotzabasis, K. Lyka, A cell-based model for the photoacclimation and CO_2-acclimation of the photosynthetic apparatus, Biochim. Biophys. Acta 1708 (2005) 250–261.
[47] E. Balestreri, P. Cioni, A. Romagnoli, S. Bernini, A. Fissi, R. Felicioli, Mechanism of polyamine inhibition of a leaf protease, Arch. Biochem. Biophys. 255 (1987) 460–463.
[48] M.E. Goldman, L. Cregar, D. Nguyen, O. Simo, S. O'Malley, T. Humphreys, Cationic polyamines inhibit anthrax lethal factor protease, BMC Pharmacol. 6 (2006) 8.
[49] M. Della Mea, A. Di Sandro, L. Dondini, S. Del Duca, F. Vantini, C. Bergamini, R. Bassi, D. Serafini-Fracassini, A Zea mays 39-kDa thylakoid transglutaminase catalyses the modification by polyamines of light-harvesting complex II in a light dependent way, Planta 219 (2004) 754–764.
[50] S. Caffari, R. Croce, L. Cattivelli, R. Bassi, A look within LHCII: differential analysis of the Lhcb1–3 complexes building the major trimeric antenna complex of higher plant photosynthesis, Biochemistry 43 (2004) 9467–9476.
[51] S. Caffari, S. Frigerio, E. Olivieri, P.G. Righetti, R. Bassi, Differential accumulation of Lhcb gene products in thylakoid membranes of Zea mays plants grown under contrasting light and temperature conditions, Proteomics 5 (2005) 758–768.
[52] M. Griffin, R. Casadio, C.M. Bergamini, Transglutaminases: Nature's biological glues, Biochem. J. 368 (2002) 377–396.
[53] A. Bograh, Y. Gingras, H.A. Tajmir-Riahi, R. Carpentier, The effects of spermine and spermidine on the structure of photosystem II proteins in relation to inhibition of electron transport, FEBS Lett. 402 (1997) 41–44.
[54] G. Jackowski, P. Olkiewicz, A. Zelisko, The acclimative response of the main light-harvesting chlorophyll a/b-protein complex of photosystem II (LHCII) to elevated irradiances at the level of trimeric subunits, J. Photochem. Photobiol., B Biol. 70 (2003) 163–170.
[55] J. Standfuss, W. Kühlbrandt, The three isoforms of the light-harvesting complex II. Spectroscopic features, trimer formation and functional roles, J. Biol. Chem. 279 (2004) 36884–36891.
[56] A.M. Timperio, L. Zolla, Investigation of the lateral light-induced migration of photosystem II Light-harvesting proteins by nano-high performance liquid chromatography electrospray ionization mass spectroscopy, J. Biol. Chem. 280 (2005) 28858–28866.