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ORIGINAL PAPER

Starch and sugar accumulation in *Sulla carnosa* leaves upon Mg^{2+} starvation

Nèjia Farhat · Mokded Rabhi · Marianna Krol · Zouhaier Barhoumi · Alexander G. Ivanov · Avery McCarthy · Chedly Abdelly · Abderrazak Smaoui · Norman P. A. Hüner

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Abstract In the present work, magnesium deficiency effects were studied in *Sulla carnosa* plants grown in nutrient solution containing 1.50, 0.05, 0.01, and 0.00 mM Mg^{2+}. After 5 weeks of treatment, fully expanded leaves were harvested to study their morphological and ultrastructural changes, as well as their carbohydrate, pigment, and Mg^{2+} concentrations. In control plants, leaves were well developed with remarkable green color. Down to 0.05 mM Mg^{2+}, no chlorosis symptom was recorded, but below this concentration, mature leaves showed an appearance of interveinal chlorosis that was much more pronounced at 0.00 mM Mg^{2+} with the development of necrotic spots. Optima of chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations were observed at 0.05 and 1.50 mM Mg^{2+}; leaf magnesium concentration was severely reduced at 0.05 mM Mg^{2+}. A significant decrease in pigment concentrations was noticed at 0.01 mM Mg^{2+}, but the lowest values were recorded at 0.00 mM Mg^{2+}. Enzymatic assays showed an increase in the accumulation of soluble sugars and starch with decreasing Mg^{2+} concentration. These results were in accordance with those of ultrastructural studies that revealed a marked alteration of chloroplasts in leaves of deficient plants. These chloroplasts were round and bigger as a result of a massive accumulation of oversized starch grains with disrupted thylakoids. As a whole, 1.50, 0.05, and 0.01 mM Mg^{2+} were found optimal, suboptimal, and deficient concentrations, respectively, the latter showing no significant difference with absolute Mg^{2+} absence (0.00 mM Mg^{2+}).

Keywords Deficient concentration · Optimal concentration · Suboptimal concentration · Soluble sugars · Starch

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N. Farhat · M. Rabhi (✉) · Z. Barhoumi · C. Abdelly · A. Smaoui
Laboratory of Extremophile Plants, Biotechnology Centre of Borj Cedria, P. O. Box 901, 2050 Hammam-Lif, Tunisia
e-mail: mokded.rabhi@gmail.com

M. Krol · A. G. Ivanov · A. McCarthy · N. P. A. Hüner
Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada

Introduction

Plants need magnesium in harvesting solar energy and in driving photochemistry (Hermans et al. 2010). Moreover, it was established that magnesium is a crucial component in plant cells since it is involved in the activation of more enzymes than any other mineral nutrient (Epstein and Bloom 2004). Among Mg-activated enzymes, the following are included: ATPases, RNA polymerase, 1,5-biphosphate carboxylase/oxidase (Rubisco), and protein kinases (Marschner 1995; Shaul 2002). In addition, being a central atom in the chlorophyll molecule (Beale 1999), magnesium plays a key role in the utilization of light energy in photosynthesis (Cakmak and Kirkby 2008). Plants suffering from magnesium deficiency constitute a worldwide problem (Mitchell et al. 1999). It was revealed that before any change in shoot growth, photosynthetic activity, and leaf morphology, the most harmful effect of magnesium starvation is sugar accumulation in source leaves (Cakmak et al. 1994a, b; Hermans and Verbruggen 2005). Therefore, this accumulation of sugar was more related to magnesium availability decline than to chlorophyll content decrease (Hermans et al. 2004; Hermans and Verbruggen 2005).

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Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophilic and a mesophilic alga

Rachael Morgan-Kiss [a], Alexander G. Ivanov [b], John Williams [c], Mobashsher Khan [c], Norman P.A. Huner [b,*]

[a] *Department of Microbiology, University of Illinois, Urbana, IL 61801, USA*
[b] *Department of Plant Sciences, University of Western Ontario, London, ON, Canada N6A 5B7*
[c] *Department of Botany, University of Toronto, Toronto, ON, Canada*

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Abstract

Sensitivity of the photosynthetic thylakoid membranes to thermal stress was investigated in the psychrophilic Antarctic alga *Chlamydomonas subcaudata*. *C. subcaudata* thylakoids exhibited an elevated heat sensitivity as indicated by a temperature-induced rise in F_o fluorescence in comparison with the mesophilic species, *Chlamydomonas reinhardtii*. This was accompanied by a loss of structural stability of the photosystem (PS) II core complex and functional changes at the level of PSI in *C. reinhardtii*, but not in *C. subcaudata*. Lastly, *C. subcaudata* exhibited an increase in unsaturated fatty acid content of membrane lipids in combination with unique fatty acid species. The relationship between lipid unsaturation and the functioning of the photosynthetic apparatus under elevated temperatures is discussed.

Keywords: Chloroplast membrane; Chlorophyll fluorescence; Heat stress; Lipid unsaturation; Photochemical apparatus; *Chlamydomonas subcaudata*

1. Introduction

The inhibitory effects of moderate to high temperatures in plants have been well documented [1], and the photosynthetic process is one of the most thermosensitive functions in the plant. While some of the causes underlying thermosensitivity in photosynthetic organisms still remain unclear, it has been well documented in plants [2,3] and algae [4,5] that the phenomenon of high temperature-induced sensitivity at the level of the thylakoid chlorophyll–protein complexes can be monitored indirectly via changes in specific chlorophyll fluorescence parameters. In particular, temperature-induced structural modulations

Abbreviations: ΔA_{820}, change in absorbance at 820 nm; ΔH, activation energy; Chl, chlorophyll; CPa, photosystem II core complex; CP1, photosystem I core complex; P_{700}, photosystem I reaction center; DBMIB, 2,5-dibromo-3-, methyl-6-isopropyl-*p*-benzoquinone; DGDG, digalactosyldiacylglycerol; $F_{688,699,700,715,722}$, 77 K fluorescence emission maxima at the respective wavelengths; FAME, fatty acid methyl ester(s); F_o, Chl *a* fluorescence of open reaction centers; F_q, maximal fluorescence yield in the absence of cations; FR, far red light; I_U, index of unsaturation; LHC, light harvesting complex; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; PS, photosystem; SQDG, sulfoquinovosyldiacylglycerol; *R*, distance between PSII and PSI chlorophyll–protein complexes; T_{CRIT}, critical temperature for maximum chlorophyll fluorescence

* Corresponding author. Fax: 519-661-3935.
E-mail address: nhuner@julian.uwo.ca (N.P.A. Huner).

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Psychrophily is associated with differential energy partitioning, photosystem stoichiometry and polypeptide phosphorylation in *Chlamydomonas raudensis*

Beth Szyszka, Alexander G. Ivanov, Norman P.A. Hüner *

Department of Biology and The Biotron, University of Western Ontario, 1151 Richmond St. N., London, Ontario, Canada N6A 5B7

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Abstract

Chlamydomonas raudensis UWO 241 and SAG 49.72 represent the psychrophilic and mesophilic strains of this green algal species. This novel discovery was exploited to assess the role of psychrophily in photoacclimation to growth temperature and growth irradiance. At their optimal growth temperatures of 8 °C and 28 °C respectively, UWO 241 and SAG 49.72 maintained comparable photostasis, that is energy balance, as measured by PSII excitation pressure. Although UWO 241 exhibited higher excitation pressure, measured as 1-qL, at all growth light intensities, the relative changes in 1-qL were similar to that of SAG 49.72 in response to growth light. In response to suboptimal temperatures and increased growth irradiance, SAG 49.72 favoured energy partitioning of excess excitation energy through inducible, down regulatory processes (Φ_{NPQ}) associated with the xanthophyll cycle and antenna quenching, while UWO 241 favoured xanthophyll cycle-independent energy partitioning through constitutive processes involved in energy dissipation (Φ_{NO}). In contrast to SAG 49.72, an elevation in growth temperature induced an increase in PSI/PSII stoichiometry in UWO 241. Furthermore, SAG 49.72 showed typical threonine-phosphorylation of LHCII, whereas UWO 241 exhibited phosphorylation of polypeptides of comparable molecular mass to PSI reaction centres but the absence of LHCII phosphorylation. Thus, although both strains maintain an energy balance irrespective of their differences in optimal growth temperatures, the mechanisms used to maintain photostasis were distinct. We conclude that psychrophily in *C. raudensis* is complex and appears to involve differential energy partitioning, photosystem stoichiometry and polypeptide phosphorylation.

Keywords: *Chlamydomonas raudensis*; Chlorophyll fluorescence; Cold acclimation; Energy partitioning; Photostasis; Psychrophily

1. Introduction

Chlamydomonas raudensis Ettl. (UWO 241) was isolated from the lowest trophic zone of Antarctica's permanently ice-covered Lake Bonney [1]. This environment is characterized by irradiance levels that do not exceed 50 μmol photons m^{-2} s^{-1}, temperatures ranging from 4 °C to 6 °C and a narrow spectral distribution enriched in the blue-green region [2]. Therefore, this unique green alga has adapted to extremely stable conditions of low light and low temperature.

Recently, UWO 241 was identified as a psychrophilic strain of *C. raudensis Ettl.* (SAG 49.72), a mesophile which originates from a meadow pool near Rudná, Nordmähren, Czech Republic [3]. Psychrophiles ("cold-loving") are organisms which exhibit optimal growth temperatures below 15 °C but are unable to grow at temperatures above 20 °C [5]. In contrast, mesophiles exhibit the capacity to grow at both high and low temperatures but exhibit optimal growth temperatures in the range of 25° to 30 °C [5]. Previous comparative studies of *C. raudensis* UWO 241 were performed using the model mesophile, *Chlamydomonas reinhardtii*, a different *Chlamydomonas* algal species.

Abbreviations: F_o and F_m, fluorescence intensity measured when all photosystem II reaction centers are open or closed, respectively; F_s, steady-state fluorescence; LHCII, light-harvesting complex II; Φ_{NO}, constitutive processes involved in energy dissipation; Φ_{NPQ}, yield of zeaxanthin-dependent non-photochemical dissipation; PQ, plastoquinone; Φ_{PSII}, yield of photochemistry; Q_A, first stable quinone electron acceptor of photosystem II; qL, relative reduction state of Q_A

* Corresponding author. Tel.: +1 519 661 2111x86488; fax: +1 519 661 3935.
E-mail address: nhuner@uwo.ca (N.P.A. Hüner).

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The induction of CP43′ by iron-stress in *Synechococcus* sp. PCC 7942 is associated with carotenoid accumulation and enhanced fatty acid unsaturation

Alexander G. Ivanov [a,b], Marianna Krol [a], Eva Selstam [b], Prafullachandra Vishnu Sane [c], Dmitry Sveshnikov [b], Youn-Il Park [d], Gunnar Öquist [b], Norman P.A. Huner [a,*]

[a] *Department of Biology and The Biotron, University of Western Ontario, 1151 Richmond Street N., London, Ontario, Canada N6A 5B7*
[b] *Department of Plant Physiology, University of Umeå, Umeå S-901 87, Sweden*
[c] *Jain Irrigation Systems Limited, Jain Hills, Jalgaon, 425001, India*
[d] *Department of Biology, Chungnam National University, Daejon 305-764, Korea*

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Abstract

Comparative lipid analysis demonstrated reduced amount of PG (50%) and lower ratio of MGDG/DGDG in iron-stressed *Synechococcus* sp. PCC 7942 cells compared to cells grown under iron sufficient conditions. In parallel, the monoenoic (C:1) fatty acids in MGDG, DGDG and PG increased from 46.8%, 43.7% and 45.6%, respectively in control cells to 51.6%, 48.8% and 48.7%, respectively in iron-stressed cells. This suggests increased membrane dynamics, which may facilitate the diffusion of PQ and keep the PQ pool in relatively more oxidized state in iron-stressed compared to control cells. This was confirmed by chlorophyll fluorescence and thermoluminescence measurements. Analysis of carotenoid composition demonstrated that the induction of isiA (CP43′) protein in response to iron stress is accompanied by significant increase of the relative abundance of all carotenoids. The quantity of carotenoids calculated on a Chl basis increased differentially with nostoxanthin, cryptoxanthin, zeaxanthin and β-carotene showing 2.6-, 3.1-, 1.9- and 1.9-fold increases, respectively, while the relative amount of caloxanthin was increased only by 30%. HPLC analyses of the pigment composition of Chl–protein complexes separated by non-denaturating SDS-PAGE demonstrated even higher relative carotenoids content, especially of cryptoxanthin, in trimer and monomer PSI Chl–protein complexes co-migrating with CP43′ from iron-stressed cells than in PSI complexes from control cells where CP43′ is not present. This implies a carotenoid-binding role for the CP43′ protein which supports our previous suggestion for effective energy quenching and photoprotective role of CP43′ protein in cyanobacteria under iron stress.

Keywords: Cyanobacteria; Carotenoid; Chl–protein complex CP43′; Iron stress; Lipid; Thermoluminescence

1. Introduction

The low solubility of iron at physiological pH limits Fe^{++} availability to aquatic autotrophs in aerobic environments, thus causing moderate to severe iron deficiency which can limit primary productivity in some aquatic ecosystems [1]. In cyanobacteria, the most abundant (approximately 10^{27} cells for the genus *Synechococcus*) photoautotrophs on Earth, the photosynthetic apparatus represents one of the most iron-enriched (22–23 atoms) cellular systems [2–4], and hence highly vulnerable to iron deficiency [3,5].

The effects of growth under Fe-deficient conditions on the structural organization of the photosynthetic apparatus [6], and functional activities of cyanobacterial cells [7–12] have been well established and reviewed [4,13–15]. Since the main target within the photosynthetic apparatus under iron deficiency is the most Fe-abundant PSI reaction center (12 irons) along with its terminal electron acceptor ferredoxin (2 irons) [2,4], iron stress

Abbreviations: AL, actinic light; Chl *a*, chlorophyll *a*; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; PSI, photosystem I; PSII, photosystem II; PQ, plastoquinone; SQDG, sulfoquinovosyldiacylglycerol

* Corresponding author. Tel.: +1 519 661 2111x86488; fax. +1 519 661 3935.
E-mail address: nhuner@uwo.ca (N.P.A. Huner).

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Review

Flexibility in photosynthetic electron transport: The physiological role of plastoquinol terminal oxidase (PTOX)☆

Allison E. McDonald [a,*], Alex G. Ivanov [b], Rainer Bode [b], Denis P. Maxwell [b], Steven R. Rodermel [c], Norman P.A. Hüner [b]

[a] Department of Biology, Wilfrid Laurier University, Science Building, 75 University Avenue West, Waterloo, Ontario, Canada N2L 3C5
[b] Department of Biology and The Biotron, The University of Western Ontario, 1151 Richmond Street N., London, Ontario, Canada N6A 5B7
[c] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA

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ABSTRACT

Oxygenic photosynthesis depends on a highly conserved electron transport system, which must be particularly dynamic in its response to environmental and physiological changes, in order to avoid an excess of excitation energy and subsequent oxidative damage. Apart from cyclic electron flow around PSII and around PSI, several alternative electron transport pathways exist including a plastoquinol terminal oxidase (PTOX) that mediates electron flow from plastoquinol to O_2. The existence of PTOX was first hypothesized in 1982 and this was verified years later based on the discovery of a non-heme, di-iron carboxylate protein localized to thylakoid membranes that displayed sequence similarity to the mitochondrial alternative oxidase. The absence of this protein renders higher plants susceptible to excitation pressure dependant variegation combined with impaired carotenoid synthesis. Chloroplasts, as well as other plastids (i.e. etioplasts, amyloplasts and chromoplasts), fail to assemble organized internal membrane structures correctly, when exposed to high excitation pressure early in development. While the role of PTOX in plastid development is established, its physiological role under stress conditions remains equivocal and we postulate that it serves as an alternative electron sink under conditions where the acceptor side of PSI is limited. The aim of this review is to provide an overview of the past achievements in this field and to offer directions for future investigative efforts. Plastoquinol terminal oxidase (PTOX) is involved in an alternative electron transport pathway that mediates electron flow from plastoquinol to O_2. This article is part of a Special Issue entitled: Regulation of Electron Transport in Chloroplasts.

1. Introduction

The thylakoid membranes of plants, green algae and cyanobacteria contain specialized photosystems that absorb and trap light energy which is subsequently transformed into chemical energy in the form of ATP and NADPH and utilized in the biochemical reduction of carbon, nitrogen, and sulfur (Fig. 1A). The photochemical reactions taking place in the photosystems are dependent upon the photophysical processes of light absorption and energy transfer within the light harvesting complex and the subsequent excitation migration from the light harvesting pigments, through the core antenna to the reaction center pigments, P680 and P700 of PSII and PSI respectively [1] (Fig. 1A). In addition to PSI and PSII, linear electron transport (LET) is comprised of a third supramolecular complex Cyt b_6f, which connects the release of electrons by PSII through photo-oxidation of P680 to the reduction of photo-chemically-derived $P700^+$ (Fig. 1A). Electrons from Q_A^- are transferred via Q_B and convert plastoquinone (PQ) to plastoquinol (PQH_2) which is subsequently oxidized by the Cyt b_6f complex (Fig. 1A). This is considered to be the rate-limiting step in photosynthetic electron transport, because it depends on the rate diffusion of PQ/PQH_2 through the thylakoid membrane [1]. $P700^+$ oxidizes the Cyt b_6f complex via plastocyanin which converts $P700^+$ to P700. The oxidation of PQH_2 via the Cyt b_6f complex occurs concomitantly with the vectorial transport of protons from the stroma to the thylakoid lumen. In the case of PSII, $P680^+$ exhibits a sufficiently positive reduction potential to oxidize H_2O via the lumenal oxygen evolving complex (Fig. 1A). This results in the reduction of $P680^+$ to P680, the evolution of O_2 and the release of protons into the thylakoid lumen. The proton gradient generated by LET is used for the chemiosmotic synthesis of ATP by the chloroplast H^+-dependent ATP synthase.

Abbreviations: ARTO, alternative respiratory terminal oxidase; CET, cyclic electron transport; COX, cytochrome c oxidase; cyt bd, bd-quinol oxidase; Cyt b_6f, cytochrome b_6f complex; DOX, diiron carboxylate protein; IM, immutans; LET, linear electron transport; NDH, chloroplastic NAD(P)H dehydrogenase complex; PET, photosynthetic electron transport; PSI, photosystem I; PSII, photosystem II; PQ, plastoquinone; PQH_2, plastoquinol; PTOX, plastoquinol terminal oxidase; QOX, bo-quinol oxidase; Rubisco, Ribulose-1,5-bisphosphate carboxylase oxygenase

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* Corresponding author. Tel.: +1 519 884 0710 x4180; fax: +1 519 746 0677.
E-mail addresses: amcdonald@wlu.ca (A.E. McDonald), aivanov@uwo.ca (A.G. Ivanov), rbode2@uwo.ca (R. Bode), dmaxwell@uwo.ca (D.P. Maxwell), rodermel@iastate.edu (S.R. Rodermel), nhuner@uwo.ca (N.P.A. Hüner).

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Restricted capacity for PSI-dependent cyclic electron flow in Δ*petE* mutant compromises the ability for acclimation to iron stress in *Synechococcus* sp. PCC 7942 cells ☆

A.G. Ivanov [a,b], P.V. Sane [c], I. Simidjiev [b], Y.-I. Park [d], N.P.A. Huner [a,*], G. Öquist [b]

[a] Department of Biology, University of Western Ontario, London, ON, Canada N6A 5B7
[b] Umeå Plant Science Center, Umeå University, Umeå, Sweden
[c] Jain Irrigation Systems Limited, Jain Hills, Jalgaon, 425001, India
[d] Department of Biological Sciences, Chungnam National University, Daejon 305-764, Republic of Korea

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ABSTRACT

Exposure of wild type (WT) and plastocyanin coding *petE* gene deficient mutant (Δ*petE*) of *Synechococcus* cells to low iron growth conditions was accompanied by similar iron-stress induced blue-shift of the main red Chl *a* absorption peak and a gradual decrease of the Phc/Chl ratio, although Δ*petE* mutant was more sensitive when exposed to iron deficient conditions. Despite comparable iron stress induced phenotypic changes, the inactivation of *petE* gene expression was accompanied with a significant reduction of the growth rates compared to WT cells. To examine the photosynthetic electron fluxes *in vivo*, far-red light induced P700 redox state transients at 820 nm of WT and Δ*petE* mutant cells grown under iron sufficient and iron deficient conditions were compared. The extent of the absorbance change ($\Delta A_{820}/A_{820}$) used for quantitative estimation of photooxidizable $P700^+$ indicated a 2-fold lower level of $P700^+$ in Δ*petE* compared to WT cells under control conditions. This was accompanied by a 2-fold slower re-reduction rate of $P700^+$ in the Δ*petE* indicating a lower capacity for cyclic electron flow around PSI in the cells lacking plastocyanin. Thermoluminescence (TL) measurements did not reveal significant differences in PSII photochemistry between control WT and Δ*petE* cells. However, exposure to iron stress induced a 4.5 times lower level of $P700^+$, 2-fold faster re-reduction rate of $P700^+$ and a temperature shift of the TL peak corresponding to $S_2/S_3Q_B^-$ charge recombination in WT cells. In contrast, the iron-stressed Δ*petE* mutant exhibited only a 40% decrease of $P700^+$ and no significant temperature shift in $S_2/S_3Q_B^-$ charge recombination. The role of mobile electron carriers in modulating the photosynthetic electron fluxes and physiological acclimation of cyanobacteria to low iron conditions is discussed. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

The photosynthetic apparatus represents one of the most iron enriched cellular systems, requiring 22–23 atoms of iron in a single copy of the linear electron transport chain in oxygenic photoautotrophs [1–3]. This makes phytoplankton highly sensitive to the limited availability of iron and can significantly reduce the global primary productivity in aquatic ecosystems [4–6]. Diverse effects of Fe-limitation on the growth of various cyanobacterial strains have been well established and reviewed [2,7,8]. Cyanobacteria respond to limited iron availability by reducing the amount of phycocyanin (Phc) and Chl *a* per cell [9,10] and the number of iron-containing proteins within the photosynthetic apparatus [11]. A large decrease in the number of thylakoid membranes and phycobilisomes [12], a blue shift of 5–6 nm in the main red absorption band of Chl *a* [9,13] and the appearance of the iron-stress induced Chl–protein complex CP43′ associated with the *isiA* gene product [14,15,7] forming a characteristic antenna ring of 18 CP43′ molecules around trimeric PSI [16,17] are distinct characteristics of iron-stressed cyanobacteria. Although *in vitro* data indicated that the CP43′ ring structure associated with PSI under Fe limitation acts to enhance the light harvesting efficiency of PSI [16,17], *in vivo* measurements of PSI absorption cross-section failed to confirm the *in vitro* measurements

Abbreviations: AC, actinic light; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; MOPS, 3-(N-morpholino) propane sulfonic acid; P700, reaction center pigment of PSI; $P700^+$, oxidized form of the reaction center pigment of PSI; PC, plastocyanin; PSI, Photosystem I; PSII, Photosystem II; Phc, phycocyanin; PQ, plastoquinone; Q_A, primary electron-accepting quinone in PSII reaction centers; TL, thermoluminescence; T_M, temperature of maximum thermoluminescence emission

☆ This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

* Corresponding author at: Department of Biology and The Biotron Center for Experimental Climate Change Research, University of Western Ontario, 1151 Richmond Street N., London, ON, Canada N6A 5B7. Tel.: +1 519 661 2111x86488; fax: + (519) 661 3935.
E-mail address: nhuner@uwo.ca (N.P.A. Huner).

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Genetic decrease in fatty acid unsaturation of phosphatidylglycerol increased photoinhibition of photosystem I at low temperature in tobacco leaves ☆

A.G. Ivanov [a,d,*], S.I. Allakhverdiev [b,c,d], N.P.A. Huner [a], N. Murata [d]

[a] Department of Biology, University of Western Ontario, London, Ontario Canada N6A 5B7
[b] Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia
[c] Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russia
[d] National Institute for Basic Biology, Okazaki 444-8585, Japan

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ABSTRACT

Leaves of transgenic tobacco plants with decreased levels of fatty acid unsaturation in phosphatidylglycerol (PG) exhibited a slightly lower level of the steady state oxidation of the photosystem I (PSI) reaction center P700 ($P700^+$) than wild-type plants. The PSI photochemistry of wild-type plants was only marginally affected by high light treatments. Surprisingly, all plants of transgenic lines exhibited much higher susceptibility to photoinhibition of PSI than wild-type plants. This was accompanied by a 2.5-fold faster re-reduction rate of $P700^+$ in the dark, indicating a higher capacity for cyclic electron flow around PSI in high light treated transgenic leaves. This was associated with a much higher intersystem electron pool size suggesting over-reduction of the PQ pool in tobacco transgenic lines with altered PG unsaturation compared to wild-type plants. The physiological role of PG unsaturation in PSI down-regulation and modulation of the capacity of PSI-dependent cyclic electron flows and distribution of excitation light energy in tobacco plants under photoinhibitory conditions at low temperatures is discussed. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

The unique and highly conserved lipid composition of thylakoid membranes is dominated by two uncharged galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG), comprising approximately 50 and 20% of the total acyl lipids in thylakoid membranes respectively. The remaining lipids are distributed between the negatively charged phosphatidylglycerol (PG) and a sulfolipid sulfoquinovosyldiacylglycerol (SQDG). PG is the phospholipid that is present in thylakoid membranes and although its amount in thylakoid membranes is only 10% of the total chloroplast lipids it accounts for approximately 85% of the total PG in plant leaves [1–4].

The essential role of acyl lipids in thylakoid membranes as one of the important factors controlling the structural organization and functional activities in photosynthetic membranes has been well characterized and reviewed [4–7]. Moreover, since PG is the only phospholipid within the thylakoid membranes, its specific role in the chloroplast development, functional activities and assembly of the photosynthetic apparatus has been extensively studied [7,3,8]. Reduced content of PG in the *pgp1* mutant of *Arabidopsis* caused retardation of plant growth and chloroplast differentiation, and a decrease in photosynthetic activity [9,10]. PG has been also identified as indispensable for photoautotrophic growth of *Synechocystis* sp. PCC 6803 [11,12]. Furthermore, the presence of PG in photosynthetic membranes in higher-plant leaves plays a significant role in modulating the susceptibility of PSII to photoinhibition and acclimation to low temperatures [13–15].

In addition, it has been demonstrated that the acclimation and sensitivity of various photoautotrophs to low temperature conditions depend not only on lipid composition, but also on unsaturation level of thylakoid lipids, which is accompanied by an increase in the fatty acid unsaturation. High levels of unsaturated fatty acids in chloroplast lipids are important to maintain plant growth, chloroplast structure, photosynthetic capacity/stability at low temperatures [16–18], altered the tolerance of cyanobacteria to salt stress [19,20] and stabilized the photosynthetic apparatus against photoinhibition at low temperature in transgenic tobacco plants [21]. It has been demonstrated that not only the overall unsaturation level of chloroplast lipids, but specifically the degree of unsaturation of fatty acids in PG in plastid membranes strongly correlates with the chilling sensitivity of higher plants [19,22,23]. A decreased level of fatty acid unsaturation of PG in thylakoid

Abbreviations: PSI, photosystem I; PSII, photosystem II; P700, reaction center pigment of PSI; $P700^+$, oxidized form of the reaction center pigment of PSI; PG, phosphatidylglycerol

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* Corresponding author at: Department of Biology, University of Western Ontario, 1151 Richmond Street N., London, Ontario, Canada N6A 5B7. Tel.: +1 519 661 2111x86638.
E-mail address: aivanov@uwo.ca (A.G. Ivanov).

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Segregation of Photosystems in Thylakoid Membranes as a Critical Phenomenon

Igor Rojdestvenski,* Alexander G. Ivanov,†‡ M. G. Cottam,§ Andrei Borodich,* Norman P. A. Huner,‡ and Gunnar Öquist*

*Department of Plant Physiology, Umeå University, Umeå S-90 187, Sweden; †Institute of Biophysics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria; and ‡Department of Plant Sciences and §Department of Physics and Astronomy, University of Western Ontario, London N6A 5B7, Canada

ABSTRACT The distribution of the two photosystems, PSI and PSII, in grana and stroma lamellae of the chloroplast membranes is not uniform. PSII are mainly concentrated in grana and PSI in stroma thylakoids. The dynamics and factors controlling the spatial segregation of PSI and PSII are generally not well understood, and here we address the segregation of photosystems in thylakoid membranes by means of a molecular dynamics method. The lateral segregation of photosystems was studied assuming a model comprising a two-dimensional (in-plane), two-component, many-body system with periodic boundary conditions and competing interactions between the photosystems in the thylakoid membrane. PSI and PSII are represented by particles with different values of negative charge. The pair interactions between particles include a screened Coulomb repulsive part and an exponentially decaying attractive part. The modeling results suggest a complicated phase behavior of the system, including quasi-crystalline phase of randomly distributed complexes of PSII and PSI at low ionic screening, well defined clustered state of segregated complexes at high screening, and in addition, an intermediate agglomerate phase where the photosystems tend to aggregate together without segregation. The calculations demonstrated that the ordering of photosystems within the membrane was the result of interplay between electrostatic and lipid-mediated interactions. At some values of the model parameters the segregation can be represented visually as well as by analyzing the correlation functions of the configuration.

INTRODUCTION

The chloroplasts of most photosynthetic organisms contain continuous thylakoid membrane system differentiated into granal stacks consisting of appressed thylakoid discs that are interconnected by non-appressed stromal thylakoids (Goodchild et al.; 1972; Anderson, 1999). In the thylakoid membranes two types of photosystems are embedded. Photosystems are pigment-protein complexes, which transform the energy of the light quanta into charge separation, vital for plant metabolism. Both types of photosystems differ in size (Staehelin and Arntzen, 1983), pigment and protein composition (Glazer and Melis, 1987), and charge (Barber, 1982; Chow et al., 1991).

A characteristic feature of the photosynthetic thylakoid membranes of higher plants and some green algae is the spatial separation of photosystem I (PSI) and photosystem II (PSII) within the grana and stroma lamellae. Thylakoids of grana stacks are mostly abundant in PSII complexes, while PSI complexes are predominant in stroma lamellae (Andersson and Anderson, 1980; Anderson and Andersson, 1982; Chow et al., 1991; Anderson, 1999). Such spatial separation of two types of photosystems is called lateral segregation.

Although the differentiation of the thylakoids into grana and stroma membrane regions is viewed as a morphological reflection of the non-random distribution of the PSII and PSI chlorophyll-protein complexes between appressed (grana) and non-appressed (stroma) membrane domains, the possible physiological significance of this phenomenon (Anderson, 1999) and the mechanisms controlling the lateral heterogeneity (Chow et al., 1991; Chow, 1999) are still a matter of discussion. The degree of thylakoid membrane stacking and the simultaneous lateral segregation of PSII and PSI complexes depend strongly on the environmental conditions in vivo (Anderson, 1999). It has been suggested that the lateral heterogeneity and the formation of grana serve the purpose of physical separation of slow (PSII) and fast (PSI) photosystems allowing the regulation of the distribution of excitation energy over the two photosystems (Anderson, 1982; Trissl and Wilhelm, 1993). More recently, grana stacking was hypothesized to play an important role in protecting PSII under condition of sustained high light irradiance (Anderson and Aro, 1994).

The phenomenon of grana formation proper is not within the scope of our paper. However, the topology of the grana disks is likely to be of importance when the grana formation proper is studied. For the formation of the disks themselves a spontaneous breaking of translational symmetry in the lateral distribution of the protein complexes in the membrane is needed. In Barber (1982) and Stys (1995) it is also discussed that the heterogeneity of protein distribution serves as the necessary condition for grana formation. Some experiments suggest (Wollman and Diner, 1980; Rubin et al., 1981) that segregation of proteins in thylakoid membrane and lamella stacking are consequent events in grana

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Address reprint requests to Dr. I. Rojdestvenski, Department of Plant Physiology, Umeå University, S-90187, Sweden. Tel.: 46-70-7195291; Fax: 46-90-7866676; E-mail: igor.rojdestvenski@plantphys.umu.se.

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Temperature/light dependent development of selective resistance to photoinhibition of photosystem I

A.G. Ivanov[a,b], R.M. Morgan[a], G.R. Gray[c], M.Y. Velitchkova[b], N.P.A. Huner[a,*]

[a] *Department of Plant Sciences, University of Western Ontario, 1151 Richmond Street N., London, Ontario, N6H 5B7, Canada*
[b] *Institute of Biophysics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[c] *MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312, USA*

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Abstract Exposure of winter rye leaves grown at 20°C and an irradiance of either 50 or 250 μmol m^{-2} s^{-1} to high light stress (1600 μmol m^{-2} s^{-1}, 4 h) at 5°C resulted in photoinhibition of PSI measured in vivo as a 34% and 31% decrease in $\Delta A_{820}/A_{820}$ ($P700^{+}$). The same effect was registered in plants grown at 5°C and 50 μmol m^{-2} s^{-1}. This was accompanied by a parallel degradation of the PsaA/PsaB heterodimer, increase of the intersystem e^{-} pool size as well as inhibition of PSII photochemistry measured as F_v/F_m. Surprisingly, plants acclimated to high light (800 μmol m^{-2} s^{-1}) or to 5°C and moderate light (250 μmol m^{-2} s^{-1}) were fully resistant to photoinhibition of PSI and did not exhibit any measurable changes at the level of PSI heterodimer abundance and intersystem e^{-} pool size, although PSII photochemistry was reduced to 66% and 64% respectively. Thus, we show for the first time that PSI, unlike PSII, becomes completely resistant to photoinhibition when plants are acclimated to either 20°C/800 μmol m^{-2} s^{-1} or 5°C/ 250 μmol m^{-2} s^{-1} as a response to growth at elevated excitation pressure. The role of temperature/light dependent acclimation in the induction of selective tolerance to PSI photoinactivation is discussed.

Key words: Photosystem I; Photoinhibition; P700; Intersystem electron pool; Excitation pressure; Low temperature

1. Introduction

Photoinhibition has been defined as a decrease of photosynthetic efficiency under light conditions that exceed the photon requirement for photosynthesis [1,2]. It is well established that low temperatures in combination with moderate or even low photon fluency rates increase the susceptibility of photosynthesis to photoinhibition [3,4]. Until recently, PSII has been generally thought to be the primary target for photoinhibition although photoinactivation of PSI was also reported in in vitro systems [5–7]. Since the initial reports on PSI photoinhibition in vivo in algal systems [8,9] and intact spinach leaves [10], there have been several additional reports of selective inhibition of PSI related photochemical activities under either weak illumination in chilling sensitive plants [11–13] or under high light in potato leaves [14]. In these reports, it was demonstrated that during light stress at low temperatures the maximum quantum yield of electron transport through PSI, the pool of photooxidizeable reaction center pigment of PSI (P700), and the efficiency of P700 oxidation were dramatically reduced [11–14]. Decreased levels of EPR-detectable $P700^{+}$ [10] and inhibition of PSI electron transport [15,16] were also shown to occur in vivo under high light at ambient temperatures. Selective photoinactivation of PSI in isolated thylakoid membranes has been also reported at chilling temperatures and at 25°C in thylakoids isolated from either chilling sensitive or chilling tolerant plants [17]. Chilling and concomitant oxidative stress have been implicated as major requirements for the PSI photoinactivation in vivo [18]. The extent of photoinhibition of PSI was also shown to be highly dependent on the growth irradiance of plants [13].

It has been proposed that the photoinhibitory process in the reaction center of PSI involves at least three steps [12,18]: (1) inactivation of the acceptor site, (2) subsequent destruction of the reaction center and (3) specific degradation of the PsaB gene product, one of the two subunits of the PSI heterodimer [19]. The primary site of the photoinactivation of PSI at chilling temperatures in vivo appears to be the iron-sulfur centers [20]. More recently, excessive linear electron transport has been suggested to be a primary cause of the loss in PSI activity [16].

It has been documented that the susceptibility of photosynthesis to photoinhibition strongly depends on the growth temperature and growth light regimes to which the organism is exposed [4,21–23]. The development of tolerance to photoinhibition of PSII has been reported for organisms grown under elevated excitation pressure estimated as $1-qP$, where qP is the coefficient of photochemical quenching. The enhanced tolerance to photoinhibition of PSII in vivo in winter wheat and winter rye appears to be primarily a consequence of an enhanced capacity to utilize the absorbed light energy through photosynthesis and ultimately growth, with minimal changes in either pigment content and composition, Lhcb abundance or leaf absorbance, whereas in the green alga *Chlorella vulgaris*, it appears to be primarily due to a reduction in light harvesting capacity coupled with an enhanced capacity to dissipate excess light non-photochemically [21–23].

To date, little or no information has been published regarding the potential influence of various temperature/light growth regimes on the development of either tolerance or susceptibility of PSI to excessive radiation. In this report, we examine this possibility and show for the first time that photosynthetic

*Corresponding author. Fax: +1 (519) 661-3935.
E-mail: nhuner@julian.uwo.ca

Abbreviations: EPR, electron paramagnetic resonance; F_m, maximal chlorophyll fluorescence with all PSII reaction centers closed in dark-adapted state; F_o, minimal fluorescence with all PSII reaction centers open in dark-adapted state; F_v, variable fluorescence (F_m-F_o); PsaA/PsaB, heterodimer of PSI reaction center; PSI, PSII, photosystem I and photosystem II, respectively; P700, reaction center pigment of PSI; $P700^{+}$, oxidized form of the reaction center pigment of PSI; qP, photochemical quenching of chlorophyll fluorescence

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Iron stress restricts photosynthetic intersystem electron transport in *Synechococcus* sp. PCC 7942

A.G. Ivanov[a,d], Y.-I. Park[b], E. Miskiewicz[a], J.A. Raven[c], N.P.A. Huner[a], G. Öquist[d,*]

[a] *Department of Plant Sciences, University of Western Ontario, London, Ont., Canada N6A 5B7*
[b] *Department of Biology, Chungnam National University, Taejon 305764, South Korea*
[c] *Department of Biological Science, University of Dundee, Dundee DD1 4HN, UK*
[d] *Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden*

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Abstract Although exposure of *Synechococcus* sp. PCC 7942 to iron stress induced the accumulation of the *isiA* gene product (CP43′) compared with non-stressed controls, immunodetection of the N-terminus of cytochrome (Cyt) *f* indicated that iron stress not only reduced the content of the 40 kDa, heme-binding, Cyt *f* polypeptide by 32% but it also specifically induced the accumulation of a new, 23 kDa, non-heme-binding, putative Cyt *f* polypeptide. Concomitantly, iron stress restricted intersystem electron transport based on the in vivo reduction of $P700^+$, monitored as $\Delta A_{820}/A_{820}$ in the presence and absence of electron transport inhibitors, as well as the inhibition of the Emerson enhancement effect on O_2 evolution. However, iron stress appeared to be associated with enhanced rates of PS I cyclic electron transport, low rates of PS I-driven photoreduction of $NADP^+$ but comparable rates for PS II+PS I photoreduction of $NADP^+$ relative to controls. We hypothesize that *Synechococcus* sp. PCC 7942 exhibits a dynamic capacity to uncouple PS II and PS I electron transport, which may allow for the higher than expected growth rates observed during iron stress.

Key words: Electron transport; Iron stress; Photosystem I; *Synechococcus* sp. PCC 7942

1. Introduction

The photosynthetic apparatus represents one of the most iron-enriched (22 or 23 atoms) cellular systems [2–4], and hence highly vulnerable to iron stress [1,2]. In response to limited iron supply, the number of iron-containing proteins within the photosynthetic apparatus is reduced [3]. Iron stress also inhibits photosystem II (PS II) photochemistry [4], the amount of photooxidizable reaction center pigment of photosystem I (PS I) (P700) [5] and the partial reaction rates associated with PS II and PS I, respectively [3]. Concomitantly, a large decrease in the amount of phycocyanin (Phc) and Chl *a* [3] accompanied by structural alterations of the thylakoid membranes and phycobilisomes [6] as well as a blue shift of 5–6 nm in the main red absorption band of Chl *a* [5] are characteristic of iron-stressed cyanobacteria. The spectral shifts in iron-stressed cells are paralleled by the appearance of a specific iron-stress-induced, *isiA* gene product, the PS II chlorophyll-protein complex, CP43′ [7]. In addition, ferredoxin is replaced by flavodoxin [8]. However, since iron-stressed cyanobacteria remain viable and grow [9,10], we hypothesized that these cells must exhibit a potential to adjust metabolically to low iron conditions. In the present study, we compared the structure, function and composition of the photosynthetic electron transport system of *Synechococcus* sp. PCC 7942 under iron stress and non-stressed conditions in order to elucidate the mechanism by which this cyanobacterium adjusts to low iron conditions.

2. Materials and methods

Synechococcus sp. PCC 7942 cells were grown axenically under non-stress conditions in liquid BG-11 inorganic medium, supplemented with 10 mM 3-[*N*-morpholino]propanesulfonic acid (MOPS) (pH 7.5) and under iron-stress conditions in liquid BG-11 medium lacking Fe-citrate as described earlier [11]. Chl and Phc contents were determined using whole cell spectra according to the method of Myers et al. [12].

Maximal photosynthetic oxygen evolution (P_{max}) and the number of functional PS II centers were measured at 38°C essentially as described earlier [11]. The number of functional PS II centers was calculated by assuming that every functional PS II center produces 1 O_2 molecule for every four flashes [12]. The number of PS I reaction centers was measured from the absorbance changes at 703 nm of thylakoid suspensions as described in [5]. Two separate tungsten light sources providing illumination at 625 nm (light 2) and 675 nm (light 1) (defined by Balzers interference filters) with various intensities were used for the enhancement measurements. Quantitative expression of enhancement was presented according to [13]: $E_2 = (V_{1,2} - V_1)/V_2$, where E_2 is the increase of photosynthetic rate, $V_{1,2}$ is the photosynthetic rate when light 1 and light 2 were presented together whereas V_1 and V_2 are the photosynthetic rates produced independently by light 1 and light 2 respectively.

The redox state of P700 of samples prepared as described by Herbert et al. [14] was determined in vivo using a PAM-101-modulated fluorometer (Heinz Walz GmbH, Effeltrich, Germany) equipped with ED-800T emitter-detector and PAM-102 units following the procedure of Schreiber at al. [15] as described in detail by Ivanov et al. [16]. The redox state of P700 was evaluated as the absorbance change around 820 nm ($\Delta A_{820}/A_{820}$) in a custom-designed cuvette at the

*Corresponding author. Fax: (46)-90-7866676.
E-mail: gunnar.oquist@plantphys.umu.se

Abbreviations: Cyt, cytochrome; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol-indophenol; FR, far red light; MOPS, 3-[*N*-morpholino]propanesulfonic acid; MT, multiple turnover flash of actinic white light; MV, methyl viologen; P700, reaction center pigment of PS I; $P700^+$, oxidized form of the reaction center of PS I; PC, plastocyanin; PS I, PS II, photosystem I and photosystem II; PQ, plastoquinone; Phc, phycocyanin; P_{max}, maximal photosynthetic oxygen evolution; ST, single turnover flash of actinic white light

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Acclimation to temperature and irradiance modulates PSII charge recombination

A.G. Ivanov[a,1], P.V. Sane[a,1], M. Krol[a], G.R. Gray[b], A. Balseris[a], L.V. Savitch[c], G. Öquist[d], N.P.A. Hüner[a,*]

[a] Department of Biology and The Biotron, University of Western Ontario, London, Canada N6A 5B7
[b] Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada S7N 5A8
[c] Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada K1A OC6
[d] Umeå Plant Science Center, Department of Plant Physiology, University of Umeå, Umeå SE-90187, Sweden

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Abstract **Acclimation of wild type and the *chlorina F2* mutant of barley to either high light or low temperature results in a 2- to 3-fold increase in non-photochemical quenching which occurred independently of either energy-dependent quenching (qE), xanthophyll cycle-mediated antenna quenching or state transitions. Results of in vivo thermoluminescence measurements used to address this conundrum indicated that excitation pressure regulates the temperature gap for $S_2Q_B^-$ and $S_2Q_A^-$ charge recombinations within photosystem II reaction centers. This is discussed in terms of photoprotection through non-radiative charge recombination.**

Keywords: Acclimation; Charge recombination; Irradiance; PSII; Temperature

1. Introduction

Changes in either irradiance or temperature exacerbate the potential imbalances in energy budget due to imbalances between the rate at which photosynthetic electrons are generated through photochemistry versus the rate at which redox potential energy is utilized through reductive metabolism [1–3]. Thus, either high light or low temperature can result in comparable excitation pressure estimated as $1 - qP$ [1–3].

Although PSII is a major component regulating photosynthetic linear electron transport, it is also extremely susceptible to photoinhibition and photodamage upon exposure to excess excitation [4–6]. Non-photochemical quenching (NPQ) through xanthophyll-dependent antenna quenching [7–10] and state transitions [11,12] represent major advances in our understanding of photoprotection of PSII. However, several reports have indicated that significant levels of NPQ can not be accounted for by xanthophyll cycle-dependent, antenna quenching [7,13–17]. Chl *a* fluorescence quenching analyses [4,14–19] as well as thermoluminescence measurements [15,20,21] indicate that reaction center quenching may contribute to photoprotection of PSII [4]. We combined thermoluminescence, a very sensitive spectroscopic technique to probe charge recombination events in PSII reaction centers in vivo [22–26], with Chl *a* fluorescence induction and P700 measurements to test the hypothesis that excitation pressure modulates PSII charge recombination events in response to growth and acclimation to high light and low temperature. The results are discussed in terms of photoprotection through a non-radiative pathway for quenching within PSII reaction centers.

2. Materials and methods

2.1. Growth conditions

WT and the *F*2 mutant of barley (*Hordeum vulgare* L.) and winter rye (*Secale cereale* L.) were grown in controlled environment chambers (Conviron, Winnipeg, MB, Canada) at four different temperature/irradiance conditions (°C/μmol photons m^{-2} s^{-1}): 20/250, 20/800, 5/50, 5/250 and a relative humidity of 50%. Fully expanded second and third leaves harvested 2 h into the photoperiod were used in all experiments.

2.2. Pigment analyses

Pigments were extracted, separated and quantified by high-performance liquid chromatography as described in detail previously [27]. The epoxidation state (EPS) of the xanthophyll cycle pigments was calculated as: EPS = (V + 0.5A)/(V + A + Z). V, violaxanthin; A, antheraxanthin; Z, zeaxanthin.

2.3. SDS–PAGE and immunoblotting

Thylakoid membranes for SDS–PAGE were isolated as described in detail previously [28]. Solubilized samples containing equal amounts of protein (20 μg $lane^{-1}$) were separated on a 15% (w/v) linear polyacrylamide gel and electrophoretically transferred to nitrocellulose membranes. Immunoblots were performed with specific antibodies raised against D1 protein (PsbA), Lhcb1, Lhcb5 and PsbS (1:5000 dilutions).

2.4. Modulated chlorophyll fluorescence

Modulated chlorophyll *a* fluorescence of dark adapted leaves was measured under ambient O_2 and CO_2 with a PAM 101 chlorophyll fluorescence measuring system (Heinz Walz GmbH, Effeltrich, Germany). NPQ and $1 - qP$ were calculated as described in [3]. All fluorescence parameters were measured at the corresponding growth temperature and growth irradiance during steady-state photosynthesis.

*Corresponding author. Fax: +1 519 661 3935.
E-mail address: nhuner@uwo.ca (N.P.A. Hüner).

[1] These authors contributed equally to this research.

Abbreviations: EPS, epoxidation state; F_v/F_m, maximum photochemical efficiency of PSII in the dark adapted state; NPQ, non-photochemical quenching; qP, photochemical quenching parameter; ST, capacity for state transition calculated as $[(F'_I - F_I) - (F'_{II} - F_{II})]/(F'_I - F_I)$; T_M, thermoluminescence peak temperature

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Potential for increased photosynthetic performance and crop productivity in response to climate change: role of CBFs and gibberellic acid

Norman P. A. Hüner[1]*, Keshav Dahal[2], Leonid V. Kurepin[1], Leonid Savitch[3], Jas Singh[3], Alexander G. Ivanov[1], Khalil Kane[4] and Fathey Sarhan[4]

[1] *Biology Department and the Biotron Centre for Experimental Climate Change Research, University of Western Ontario, London, ON, Canada*
[2] *Department of Biological Sciences, University of Toronto at Scarborough, Scarborough, ON, Canada*
[3] *Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada*
[4] *Département des Sciences biologiques, Université du Québec à Montréal, Montréal, QC, Canada*

Edited by:
Matteo Balderacchi, Università Cattolica del Sacro Cuore, Italy

Reviewed by:
Peter Horton, University of Sheffield, UK
Michael Wisniewski, United States Department of Agriculture, USA

****Correspondence:***
Norman P. A. Hüner, Department of Biology, Rm 301J NCB, University of Western Ontario, North Campus Bldg., 1151 Richmond St. N., London, ON N6A 5B7, Canada
e-mail: nhuner@uwo.ca

We propose that targeting the enhanced photosynthetic performance associated with the cold acclimation of winter cultivars of rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), and *Brassica napus* L. may provide a novel approach to improve crop productivity under abiotic as well as biotic stress conditions. In support of this hypothesis, we provide the physiological, biochemical, and molecular evidence that the dwarf phenotype induced by cold acclimation is coupled to significant enhancement in photosynthetic performance, resistance to photoinhibition, and a decreased dependence on photoprotection through non-photochemical quenching which result in enhanced biomass production and ultimately increased seed yield. These system-wide changes at the levels of phenotype, physiology, and biochemistry appear to be governed by the family of C-repeat/dehydration-responsive family of transcription factors (CBF/DREB1). We relate this phenomenon to the semi-dwarf, gibberellic acid insensitive (GAI), cereal varieties developed during the "green revolution" of the early 1960s and 1970s. We suggest that genetic manipulation of the family of C-repeat/dehydration-responsive element binding transcription factors (CBF/DREB1) may provide a novel approach for the maintenance and perhaps even the enhancement of plant productivity under conditions of sub-optimal growth conditions predicted for our future climate.

Keywords: phenotypic plasticity, photosynthetic performance, crop productivity, CBFs, gibberellic acid, climate change

INTRODUCTION

The increase in the yield of major food crops since the mid-1950s has been achieved mainly through genetic improvement and increased use of agricultural inputs such as fertilizers, pesticides, and water (Murchie et al., 2009). Zhu et al. (2010) have suggested that the yield of major food crops since the last decade is increasing slowly, which may indicate that yield increase due to improved agricultural practices has reached an upper theoretical limit. Thus, it appears that the further enhancement in crop yield can only be achieved by enhancing genetic yield potential, that is, the seed yield that a crop can achieve per unit ground area under optimum growth conditions without biotic and abiotic stresses. The maximum potential biomass and grain yield that a plant can produce is determined essentially by the following five yield variables: (a) the amount of incident solar radiation available over the growing season of a plant, (b) the light interception efficiency, that is, the efficiency of the photosynthetic pigments to intercept photosynthetic active radiation, (c) the energy conversion efficiency, that is, the ratio of the biomass energy produced over a given period to the radiative energy intercepted by the canopy over the same period, (d) the translocation of photosynthates to sinks, as determined by sink strength, and (e) the partitioning efficiency, that is, the amount of total biomass energy partitioned into seed production per unit ground area, also known as harvest index (HI) (Loomis and Amthor, 1999; Long et al., 2006; Zhu et al., 2010).

Since energy partitioning efficiency and light interception efficiency have approached the theoretical upper limit (Zhu et al., 2010), further increase in yield potential can only be achieved by an increase in the energy conversion efficiency into biomass. Since plant dry matter consists of about 40% carbon by weight, an increase in total biomass production can be achieved through enhanced photosynthetic carbon assimilation (Murchie et al., 2009). Although photosynthesis is the ultimate basis for the conversion of light energy into biomass and seed yield to date, improving photosynthetic carbon assimilation has played only a minor role in enhancing energy conversion to biomass and seed yield (Long et al., 2006; Zhu et al., 2010).

This is, in part, due to the important photoprotective mechanisms that have evolved in all photoautotrophs to protect the photosynthetic apparatus from irradiance that is in excess of that which can be utilized for either reductive CO_2 assimilation as well as reductive N and S assimilation (Adams III and Demmig-Adams, 1993; Demmig-Adams and Adams III, 1996;

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Effects of low temperature stress on excitation energy partitioning and photoprotection in *Zea mays*

Leonid V. Savitch[A,D,E], *Alexander G. Ivanov*[B,D], *Loreta Gudynaite-Savitch*[B,C], *Norman P. A. Huner*[B] *and John Simmonds*[A]

[A]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre (ECORC), Central Experimental Farm, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada.
[B]Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada.
[C]Present address: Iogen Corporation, 310 Hunt Club Road East, Ottawa, ON K1V 1C1, Canada.
[D]These authors contributed equally to the writing of this manuscript.
[E]Corresponding author. Email: savitchl@agr.gc.ca

Abstract. Analysis of the partitioning of absorbed light energy within PSII into fractions utilised by PSII photochemistry (Φ_{PSII}), thermally dissipated via ΔpH- and zeaxanthin-dependent energy quenching (Φ_{NPQ}) and constitutive non-photochemical energy losses ($\Phi_{f,D}$) was performed in control and cold-stressed maize (*Zea mays* L.) leaves. The estimated energy partitioning of absorbed light to various pathways indicated that the fraction of Φ_{PSII} was twofold lower, whereas the proportion of thermally dissipated energy through Φ_{NPQ} was only 30% higher, in cold-stressed plants compared with control plants. In contrast, $\Phi_{f,D}$, the fraction of absorbed light energy dissipated by additional quenching mechanism(s), was twofold higher in cold-stressed leaves. Thermoluminescence measurements revealed that the changes in energy partitioning were accompanied by narrowing of the temperature gap (ΔT_M) between $S_{2/3}Q_B^-$ and $S_2Q_A^-$ charge recombinations in cold-stressed leaves to 8°C compared with 14.4°C in control maize plants. These observations suggest an increased probability for an alternative non-radiative $P680^+Q_A^-$ radical pair recombination pathway for energy dissipation within the reaction centre of PSII in cold-stressed maize plants. This additional quenching mechanism might play an important role in thermal energy dissipation and photoprotection when the capacity for the primary, photochemical (Φ_{PSII}) and zeaxanthin-dependent non-photochemical quenching (Φ_{NPQ}) pathways are thermodynamically restricted in maize leaves exposed to cold temperatures.

Additional keywords: cold stress, non-photochemical quenching, PSII photochemistry, thermoluminescence.

Introduction

Environmental changes in temperature, irradiance and other factors (e.g. nutrients, water availability) result in imbalances between the light energy absorbed through photochemistry and energy utilisation through photosynthetic electron transport coupled to carbon, nitrogen and sulfur reduction. It has been demonstrated and reviewed that the potential for such energy imbalance in all photosynthetic organisms is significantly increased under conditions of cold temperatures, which lead to increased PSII excitation pressure (Huner *et al*. 1996, 1998). The increased excitation pressure estimated by the chlorophyll fluorescence parameter 1 – qL (Kramer *et al*. 2004), measures the relative reduction state of Q_A, the first stable quinone electron acceptor of PSII. This, in turn, reflects the redox state of the plastoquinone pool and the intersystem electron transport chain (Gray *et al*. 1996; Huner *et al*. 1996, 1998). Such an imbalance imposed by low temperature might lead to photoinhibition of photosynthesis, which in turn might result in photodamage of the D1 reaction centre polypeptide of PSII (Krause 1988; Aro *et al*. 1993).

Low temperatures impose strong thermodynamic restrictions on CO_2 assimilation in maize (*Zea mays* L.; Greer and Hardacre 1989; Kingston-Smith *et al*. 1997; Fryer *et al*. 1998; Foyer *et al*. 2002) and this might also lead to photoinhibition of photosynthesis and photodamage of PSII (Nie *et al*. 1992; Baker 1994). The inhibitory effects of low temperature on the photosynthetic performance of maize have been extensively studied using chlorophyll fluorescence measurements under both controlled and field conditions (Havaux 1987; Krall and Edwards 1991; Andrews *et al*. 1995; Massacci *et al*.1995; Haldimann *et al*. 1996; Fracheboud *et al*. 1999; Koscielniak and Biesaga-Koscielniak 2006). As there is a close, although not always linear, correlation between CO_2 fixation and the quantum efficiency of PSII in maize (Foyer *et al*. 2002), cold-induced limitations on photosynthesis, that is, a decrease in the photochemical use of absorbed light energy, can induce the production of potentially dangerous reactive oxygen species (ROS) (Baker 1994) and would require the use of one or more mechanisms for non-photochemical dissipation of excitation light energy to prevent photodamage of PSII (Ortiz-Lopez *et al*. 1990).

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A Transient Exchange of the Photosystem II Reaction Center Protein D1:1 with D1:2 during Low Temperature Stress of *Synechococcus* sp. PCC 7942 in the Light Lowers the Redox Potential of Q_B*

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P. V. Sane‡, Alexander G. Ivanov§, Dmitry Sveshnikov‡, Norman P. A. Huner§, and Gunnar Öquist‡¶

From the ‡Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, S-90187 Umeå, Sweden and the §Department of Plant Sciences, University of Western Ontario, London, Ontario N6H 5B7 Canada

Upon exposure to low temperature under constant light conditions, the cyanobacterium *Synechococcus* sp. PCC 7942 exchanges the photosystem II reaction center D1 protein form 1 (D1:1) with D1 protein form 2 (D1:2). This exchange is only transient, and after acclimation to low temperature the cells revert back to D1:1, which is the preferred form in acclimated cells (Campbell, D., Zhou, G., Gustafsson, P., Öquist, G., and Clarke, A. K. (1995) *EMBO J.* 14, 5457–5466). In the present work we use thermoluminescence to study charge recombination events between the acceptor and donor sides of photosystem II in relation to D1 replacement. The data indicate that in cold-stressed cells exhibiting D1:2, the redox potential of Q_B becomes lower approaching that of Q_A. This was confirmed by examining the *Synechococcus* sp. PCC 7942 inactivation mutants R2S2C3 and R2K1, which possess only D1:1 or D1:2, respectively. In contrast, the recombination of Q_A^- with the S_2 and S_3 states did not show any change in their redox characteristics upon the shift from D1:1 to D1:2. We suggest that the change in redox properties of Q_B results in altered charge equilibrium in favor of Q_A. This would significantly increase the probability of Q_A^- and $P680^+$ recombination. The resulting non-radiative energy dissipation within the reaction center of PSII may serve as a highly effective protective mechanism against photodamage upon excessive excitation. The proposed reaction center quenching is an important protective mechanism because antenna and zeaxanthin cycle-dependent quenching are not present in cyanobacteria. We suggest that lowering the redox potential of Q_B by exchanging D1:1 for D1:2 imparts the increased resistance to high excitation pressure induced by exposure to either low temperature or high light.

The responses of *Synechococcus* and other unicellular cyanobacteria to various abiotic stresses have been extensively investigated and have provided useful information in understanding the mechanisms employed by cyanobacteria to overcome unfavorable environmental conditions such as chilling temperatures (1, 2), excess light (3–8), and UV-B (290–320 nm) exposure (9). It has been shown that during environmental stress and acclimation *Synechococcus* has the ability to shift between two different forms of the D1 polypeptide of the PSII[1] reaction center complex (4). D1 form 1 (D1:1) is the preferred form in a cell acclimated to its normal growth environment, but when stressed under either high light or low temperatures D1:1 is exchanged for another form of the D1 protein called D1 form 2 (D1:2) (2, 6). However, this exchange is only transient, and when cells have acclimated to the new growth conditions they revert to D1:1 (10). These shifts are governed by changes in the relative expression of the *psbAI* gene encoding for D1:1 and the *psbAII/III* genes encoding for D1:2 (2, 4).

The D1:1 product of the *psbAI* gene is different from the D1:2 product of the *psbAII/III* genes (11). Of the 25 different amino acids, 13 reside in the N-terminal part, four in helix B, three in helix C, two in helix E, and the remaining three in the C-terminal region. Both D1:1 and D1:2 have a total of 360 amino acids. Gene-inactivation mutant strains expressing either only D1:1 (R2S2C3) or only D1:2 (R2K1) (11) have proven to be very useful in studies of differential *psbA* expression in response to environmental changes (10, 12).

Cells with D1:2 appear to be more stress-resistant than those possessing D1:1 under conditions when the excitation pressure on PSII increases due to either increased irradiance or decreased temperature (2, 3, 6). This is partly due to a high rate of D1 synthesis and expression of the *psbAII/III* genes forming D1:2 under high excitation pressure (3, 4) and partly due to a higher intrinsic resistance of PSII reaction centers with D1:2 to photoinhibition (5, 13, 14).

Because the replacement of D1:1 by D1:2 is expected to modify the functional characteristics of PSII at the molecular level, we hypothesized that the D1:1 to D1:2 exchange affects PSII charge stabilization and charge recombination events as a consequence of alteration in the redox behavior of Q_A and Q_B as well as the water oxidation complex (the S states). The back reactions of Q_A and Q_B with the different S states, which reflect the charge stabilization on both acceptors and donors of PSII, were assessed by using the technique of thermoluminescence (15, 16).

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¶ To whom correspondence should be addressed. Tel.: 46-90-786-54-16; Fax: 46-90-786-66-76; E-mail: gunnar.oquist@plantphys.umu.se.

[1] The abbreviations used are: PSII, photosystem II; TL, thermoluminescence; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Q_A, primary electron-accepting quinone in PSII; Q_B, secondary electron-accepting quinone in PSII; T_M, temperature of maximum thermoluminescence emission.

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RESEARCH PAPER

Effect of cold acclimation on the photosynthetic performance of two ecotypes of *Colobanthus quitensis* (Kunth) Bartl.

León A. Bravo[1,*], Felipe A. Saavedra-Mella[1], Felipe Vera[1], Alexi Guerra[1], Lohengrin A. Cavieres[1], Alexander G. Ivanov[2], Norman P. A. Huner[2] and Luis J. Corcuera[1]

[1] *Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Correo 3, Concepción, Chile*

[2] *Department of Biology and The Biotron, University of Western Ontario, London, Ontario, Canada N6A 5B7*

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Abstract

The effects of cold acclimation of two ecotypes (Antarctic and Andes) of *Colobanthus quitensis* (Kunth) Bartl. Caryophyllaceae on their photosynthetic characteristics and performance under high light (HL) were compared. Non-acclimated plants of the Antarctic ecotype exhibited a higher (34%) maximal rate of photosynthesis than the Andes ecotype. In cold-acclimated plants the light compensation point was increased. Dark respiration was significantly increased during the exposure to 4 °C in both ecotypes. Cold-acclimated Antarctic plants showed higher Φ_{PSII} and *qP* compared with the Andes ecotype. In addition, the Antarctic ecotype exhibited higher heat dissipation (*NPQ*), especially in the cold-acclimated state, which was mainly associated with the fast relaxing component of non-photochemical quenching (NPQ_F). By contrast, the Andes ecotype exhibited a lower NPQ_F and a significant increase in the slowly relaxing component (NPQ_s) at low temperature and HL, indicating higher sensitivity to low temperature-induced photoinhibition. Although the xanthophyll cycle was fully operational in both ecotypes, cold-acclimated Antarctic plants exposed to HL exhibited higher epoxidation state of the xanthophyll cycle pigments (*EPS*) compared with the cold-acclimated Andes ecotype. Thus, the photosynthetic apparatus of the Antarctic ecotype operates more efficiently than that of the Andes one, under a combination of low temperature and HL. The ecotype differences are discussed in relation to the different climatic conditions of the two *Colobanthus*.

Key words: Antarctic plants, heat dissipation, low temperature, non-photochemical quenching, photoinhibition, photosynthesis.

Introduction

Excess irradiance may be harmful for plants that are unable to balance the absorbed/utilized energy ratio (Huner *et al*., 1998). This may be even worse when plants are exposed simultaneously to high light and low temperatures which decrease carbon and other enzymatic assimilation processes, creating a greater imbalance because light absorption is largely temperature insensitive (Huner *et al*., 1998). However, cold acclimation decreases susceptibility to photoinhibition (Krause, 1994) by causing several metabolic alterations and producing changes at the chloroplast level that may restore the energy balance. A widely accepted hypothesis is that cold acclimation may improve the ability of plants to maintain metabolism at low temperature by keeping Q_A, the

* To whom correspondence should be addressed. E-mail: lebravo@udec.cl

Abbreviations: A, antheraxanthin; *EPS*, epoxidation state of the xanthophyll cycle pigments; F_o, instantaneous (dark) chlorophyll fluorescence at open PSII centres in dark-adapted samples; F_m, maximal fluorescence at closed PSII centres; F_v, variable fluorescence; HL, high light intensity; *LCP*, light compensation point; LL, low light intensity; *NPQ*, non-photochemical quenching; NPQ_F, NPQ_s, fast and slow relaxing component of the *NPQ*; respectively; Pn_{max}, maximum rate of net photosynthesis; Φ_{PSII}, quantum yield of PSII; Φ_{O_2}, quantum yield of oxygen evolution; *qE*, energy-dependent quenching of chlorophyll fluorescence; *qI*, photoinhibitory quenching; *qP*, photochemical quenching; *Rd*, dark respiration; VAZ, pool of the xanthophyll cycle pigments; V, violaxanthin; Z, zeaxanthin.

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RESEARCH PAPER

Excess manganese differentially inhibits photosystem I versus II in *Arabidopsis thaliana*

R. Millaleo[1], M. Reyes-Díaz[2,3], M. Alberdi[2,3,*], A. G. Ivanov[4], M. Krol[4] and N. P. A. Hüner[4]

[1] Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile
[2] Departamento de Ciencias Químicas y Recursos Naturales; Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera, Temuco, Chile
[3] Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Temuco, Chile
[4] Department of Biology and The Biotron Centre for Experimental Climate Change Research, Western University, London, Ontario, N6A 5B7, Canada

* To whom correspondence should be addressed. E-mail: malberdi@ufro.cl

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Abstract

The effects of exposure to increasing manganese concentrations (50–1500 μM) from the start of the experiment on the functional performance of photosystem II (PSII) and photosystem I (PSI) and photosynthetic apparatus composition of *Arabidopsis thaliana* were compared. In agreement with earlier studies, excess Mn caused minimal changes in the PSII photochemical efficiency measured as F_v/F_m, although the characteristic peak temperature of the $S_{2/3}Q_B^-$ charge recombinations was shifted to lower temperatures at the highest Mn concentration. SDS-PAGE and immunoblot analyses also did not exhibit any significant change in the relative abundance of PSII-associated polypeptides: PSII reaction centre protein D1, Lhcb1 (major light-harvesting protein of LHCII complex), and PsbO (OEC33, a 33 kDa protein of the oxygen-evolving complex). In addition, the abundance of Rubisco also did not change with Mn treatments. However, plants grown under excess Mn exhibited increased susceptibility to PSII photoinhibition. In contrast, *in vivo* measurements of the redox transients of PSI reaction centre (P700) showed a considerable gradual decrease in the extent of P700 photooxidation ($P700^+$) under increased Mn concentrations compared to control. This was accompanied by a slower rate of $P700^+$ re-reduction indicating a downregulation of the PSI-dependent cyclic electron flow. The abundance of PSI reaction centre polypeptides (PsaA and PsaB) in plants under the highest Mn concentration was also significantly lower compared to the control. The results demonstrate for the first time that PSI is the major target of Mn toxicity within the photosynthetic apparatus of *Arabidopsis* plants. The possible involvement mechanisms of Mn toxicity targeting specifically PSI are discussed.

Key words: Chlorophyll fluorescence, Mn toxicity, photosystem I, PSI-associated proteins, PSII-associated proteins, redox state of P700.

Introduction

Manganese (Mn) is one of the most abundant metals in the Earth's crust and although it is an important essential micronutrient for all photosynthetic organisms can be also toxic when it is present in excess (Mukhopadhyay and Sharma, 1991; Marschner, 1995). Mn is considered the second most phytotoxic element, after aluminium (Al), affecting negatively the physiological and biochemical properties of plant species (Foy *et al.*, 1978, Foy, 1984; Millaleo *et al.*, 2010). An

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Protection of Photosystem II Against UV-A and UV-B Radiation in the Cyanobacterium *Plectonema boryanum*: The Role of Growth Temperature and Growth Irradiance¶

Alexander G. Ivanov[1,2], Ewa Miskiewicz[1], Adrian K. Clarke[3], Bruce M. Greenberg[4] and Norman P. A. Huner*[1]

[1]Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada;
[2]Institute of Biophysics, Bulgarian Academy of Sciences, Sofia, Bulgaria;
[3]Department of Plant Physiology, University of Umeå, Umeå, Sweden and
[4]Biology Department, University of Waterloo, Waterloo, Ontario, Canada

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ABSTRACT

***Plectonema boryanum* UTEX 485 cells were grown at 29°C and 150 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) and exposed to PAR combined with ultraviolet-A radiation (UV-A) at 15°C. This induced a time-dependent inhibition of photosystem II (PSII) photochemistry measured as a decrease of the chlorophyll *a* fluorescence ratio, F_v/F_m, to 50% after 2 h of UV-A treatment compared to nontreated control cells. Exposure of the same cells to PAR combined with UV-A + ultraviolet-B radiation (UV-B) caused only a 30% inhibition of PSII photochemistry relative to nontreated cells. In contrast, UV-A and UV-A + UV-B irradiation of cells cultured at 15°C and 150 μmol m^{-2} s^{-1} had minimal effects on the F_v/F_m values. However, cells grown at 15°C and lower PAR irradiance (6 μmol m^{-2} s^{-1}) exhibited similar inhibition patterns of PSII photochemistry as control cells. The decreased sensitivity of PSII photochemistry of *P. boryanum* grown at 15°C and 150 μmol m^{-2} s^{-1} to subsequent exposure to UV radiation relative to either control cells or cells grown at low temperature but low irradiance was correlated with the following: (1) a reduced efficiency of energy transfer to PSII reaction centers; (2) higher levels of a carotenoid tentatively identified as myxoxanthophyll; (3) the accumulation of scytonemin and mycosporine amino acids; and (4) the accumulation of ATP-dependent caseinolytic proteases. Thus, acclimation of *P. boryanum* at low temperature and moderate irradiance appears to confer significant resistance to UV-induced photoinhibition of PSII. The role of excitation pressure in the induction of this resistance to UV radiation is discussed.**

INTRODUCTION

Recent studies have indicated that exposure to ultraviolet-B radiation (UV-B)† induced photodestruction of the major cyanobacterial light harvesting complex, the phycobilisome, and each of its constituent phycobiliproteins as well as linker polypeptides (1,2). This resulted in a reduced efficiency of energy transfer from the accessory pigments to the reaction center of photosystem II (PSII) (2). UV-B exposure of a number of freshwater and marine cyanobacteria also leads to the inhibition of PSII photochemical efficiency estimated *in vivo* by the room temperature chlorophyll (Chl) *a* fluorescence emission ratio, F_v/F_m (3), whole chain photosynthetic electron transport as well as O_2 evolution (4). In addition, it has been reported that photosynthetic CO_2 fixation (4), nitrate uptake (5) and nitrogenase activity (6) were severely inhibited by UV-B treatment.

The major UV-B target sites on the acceptor-side of PSII in cyanobacteria include the depletion of the plastoquinone pool (7) as well as the degradation of the D1 protein of PSII reaction center due to the cleavage of the second transmembrane segment of the D1 polypeptide (3,7). Alternatively, a donor-side mechanism for UV-B–induced damage to PSII involving the inhibition of the water-splitting complex has been proposed as the main cleavage site of the D1 protein (7). Recently, Campbell *et al.* (3) reported that UV-B strongly regulates expression of *psbA* genes and leads to exchange of alternate PSII D1 polypeptides in *Synechococcus* sp. PCC 7942 cells. The capability to exchange the D1:1 form for the D1:2 form has been considered as one of the main mecha-

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*To whom correspondence should be addressed at: Department of Plant Sciences, University of Western Ontario, 1151 Richmond Street N., London, Ontario, Canada N6A 5B7. Fax: 519-661-3935; e-mail: nhuner@julian.uwo.ca

†*Abbreviations:* Chl, chlorophyll; Clp, ATP-dependent caseinolytic protease; F_m, maximal chlorophyll fluorescence in dark adapted state; F_0, minimal chlorophyll fluorescence in dark adapted state; F_s, steady-state fluorescence yield; F_v, variable fluorescence ($F_m - F_0$); F_v/F_m, maximal quantum efficiency of PSII; HPLC, high-performance liquid chromatography; OS-MAA, oligosaccharide-mycosporine amino acid; PAR, photosynthetically active radiation; PPFD, photosynthetic photon flux density; PSII, photosystem II; qN, non-photochemical quenching coefficient; qP, photochemical quenching coefficient; Scy, scytonemin; TES, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; UV-A, ultraviolet-A radiation; UV-B, ultraviolet-B radiation.

Photosynthesis Research **56:** 209–221, 1998.

Regular paper

Adjustment of thylakoid plastoquinone content and Photosystem I electron donor pool size in response to growth temperature and growth irradiance in winter rye (*Secale cereale* L.)

Gordon R. Gray[1], Alexander G. Ivanov, Marianna Król & Norman P.A. Huner*
Department of Plant Sciences, The University of Western Ontario, London, ON N6A 5B7, Canada; [1]*Present Address: MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312, USA;* *Author for correspondence*

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Key words: electron donor pool size, irradiance, Photosystem I, plastoquinone, temperature, stoichiometry

Abstract

Winter rye (*Secale cereale* L. cv Musketeer) grown at 5 °C/250 μmol photons m^{-2} s^{-1} exhibited a relative reduction state of PS II comparable to that of rye grown at 20 °C but high light (800 μmol photons m^{-2} s^{-1}) (1-q_P = 0.32) whereas winter rye grown at 20 °C/250 μmol photons m^{-2} s^{-1} exhibited values of 1-q_P ($\approx$ 0.15) comparable to plants grown at 5 °C but low light (50 μmol photons m^{-2} s^{-1}). The apparent size of the electron donor pool to PS I, estimated either *in vivo* or *in vitro* in the presence of methylviologen by ΔA_{820} was positively correlated with the relative reduction state of PS II under the steady-state growth conditions. Immunoblotting of rye thylakoid polypeptides indicated that the relative contents of Lhcb1, Lhcb2, D1, Cyt *f*, PC, PsaA/PsaB heterodimer and the β-subunit of ATPase complex exhibited minimal changes on a Chl basis. In contrast, a 2-fold increase in plastoquinone A content was associated with increasing growth irradiance at growth temperatures of either 5 or 20 °C. We suggest that the increases in the apparent size of the electron donor pool to PS I associated with rye grown at either 5 °C/250 μmol photons m^{-2} s^{-1}or 20 °C/800 μmol photons m^{-2} s^{-1} may be explained by an increased thylakoid plastoquinone A content, coupled with possible enhanced PS I cyclic electron transport and/or increased capacity for electron donation from the stroma to the intersystem electron transport chain. The results are discussed with respect to photosynthetic adjustment to changes in PS II 'excitation pressure' in winter rye.

Abbreviations: ΦO_2 – quantum yield of photosynthetic oxygen evolution; 1-q_P – relative reduction state of PS II; ΔA_{820} – redox state of P_{700} measured as P_{700}^+; ATPase – chloroplastic ATP-synthase (CF_0–CF_1 complex); DSC – differential scanning calorimetry; e^- – electron; F_M' – maximal fluorescence in the light-adapted state; F_O' – minimal fluorescence in the light-adapted state; FR – far-red; F_S – steady-state fluorescence yield; LED – light-emitting diode; Lhcb – Chl *a/b*-binding proteins of PS II; *Lhcb* – genes encoding Chl *a/b*-binding proteins of PS II; MT – multiple turnover flash; MV – methylviologen; P_{700} – primary electron donor of PS I; P_{700}^+ – cation radical of P_{700}; PC – plastocyanin; P_{max} – maximum photosynthetic capacity; PPFD – photosynthetic photon flux density; PQ – plastoqinone; PQA – plastoquinone A; PsaA/PsaB – PS I reaction center heterodimer; q_P – coefficient of photochemical quenching; ST – single turnover flash; $t_{1/2}$ – half-time for completion of an event

Introduction

Changes in the composition, function and organization of the photosynthetic apparatus in response to long-term changes in light quantity and quality are well documented in many plants (Anderson 1986; Wild et al. 1986; Chow et al. 1990; De la Torre and Burkey 1990; Walters and Horton 1994, 1995; Burkey and Wells 1996), green algae (Murakami et al. 1997) and cyanobacteria (Fujita et al. 1994). In general,

Photosynthesis Research **56:** 303–314, 1998.

Regular paper

Structure and composition of the photochemical apparatus of the Antarctic green alga, *Chlamydomonas subcaudata*

Rachael M. Morgan[1], Alexander G. Ivanov[1], John C. Priscu[2], Denis P. Maxwell[3] & Norman P. A. Huner[1,*]

[1] *Department of Plant Sciences, The University of Western Ontario, London, ON N6A 5B7, Canada;* [2] *Biology Department, Montana State University, Bozeman, MT 59717 USA;* [3] *MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312, USA; *Author for correspondence*

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Key words: Antarctic alga, *Chlamydomonas*, 77 K fluorescence, light harvesting, Photosystem I, psychrophile

Abstract

The green alga, *Chlamydomonas subcaudata*, collected from a perennially ice-covered Antarctic lake, was able to grow at temperatures of 16 °C or lower, but not at temperatures of 20 °C or higher, which confirmed its psychrophilic nature. Low temperature (77 K) Chl *a* fluorescence emission spectra of whole cells of the mesophile, *C. reinhardtii*, indicated the presence of major emission bands at 681 and 709 nm associated with PS II and PS I, respectively. In contrast, emission spectra of whole cells of *C. subcaudata* exhibited major emission bands at 681 and 692 nm associated with PS II, but the absence of a major PS I emission band at 709 nm. These results for *C. subcaudata* were consistent with: (1) low ratio of Chl *a/b* (1.80); (2) low levels of PsaA/PsaB heterodimer as well as specific *Lhca* polypeptides as determined by immunoblotting, (3) decreased levels of the Chl-protein complexes CP1 and LHC I associated with PS I; and (4) an increased stability of the oligomeric form of LHC II as assessed by non-denaturing gel electrophoresis in the psychrophile compared to the mesophile. Furthermore, immunoblotting indicated that the stoichiometry of PS II:PS I:CF_1 is significantly altered in *C. subcaudata* compared to the mesophile. Even though the psychrophile is adapted to growth at low irradiance, it retained the capacity to adjust the total xanthophyll cycle pool size as well as the epoxidation state of the xanthophyll cycle. Despite these differences, the psychrophile and mesophile exhibited comparable photosynthetic efficiency for O_2 evolution regardless of growth conditions. P_{max} for both *Chlamydomonas* species was similar only when grown under identical conditions. We suggest that these photosynthetic characteristics of the Antarctic psychrophile reflect the unusual light and low temperature regime to which it is adapted.

Abbreviations: α – photosynthetic efficiency; $\sigma_{PS\,I,PS\,II}$ – absorptive cross section of Photosystem I or II; BBM – Bold's basal medium: CF_1 – coupling factor 1; DOC – deoxycholic acid; $F_{678,681,692,709}$ – 77 K fluorescence emission maxima at the respective wavelengths; LHC I – light-harvesting complex I; LHC II1 – monomeric LHC II; LHC II3 – oligomeric LHC II; μ_{max} – maximum growth rate; PS I – Photosystem I; PS II – Photosystem II; P_{max} – maximum light and CO_2-saturated rate of O_2 evolution; t_{gen} – doubling time; SDS – sodium dodecyl sulfate

Introduction

Phytoplankton which inhabit polar environments are generally adapted to a variety of extreme growth conditions. Algae endemic to Antarctica typically require low temperatures for optimal growth, and are thus, classified as psychrophilic algae (Eddy 1960). Conversely, several Arctic species exhibit a broad range in growth temperatures, and thus are often not obligated to grow under a low temperature regime (Bolton and Lüning 1982). It is thought that Antarctic marine phytoplankton exhibit a requirement for low temperature

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REVIEW

Photosystem II reaction centre quenching: mechanisms and physiological role

Alexander G. Ivanov · Prafullachandra V. Sane · Vaughan Hurry · Gunnar Öquist · Norman P. A. Huner

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Abstract Dissipation of excess absorbed light energy in eukaryotic photoautotrophs through zeaxanthin- and ΔpH-dependent photosystem II antenna quenching is considered the major mechanism for non-photochemical quenching and photoprotection. However, there is mounting evidence of a zeaxanthin-independent pathway for dissipation of excess light energy based within the PSII reaction centre that may also play a significant role in photoprotection. We summarize recent reports which indicate that this enigma can be explained, in part, by the fact that PSII reaction centres can be reversibly interconverted from photochemical energy transducers that convert light into ATP and NADPH to efficient, non-photochemical energy quenchers that protect the photosynthetic apparatus from photodamage. In our opinion, reaction centre quenching complements photoprotection through antenna quenching, and dynamic regulation of photosystem II reaction centre represents a general response to any environmental condition that predisposes the accumulation of reduced Q_A in the photosystem II reaction centres of prokaryotic and eukaryotic photoautotrophs. Since the evolution of reaction centres preceded the evolution of light harvesting systems, reaction centre quenching may represent the oldest photoprotective mechanism.

Keywords Energy dissipation · Non-photochemical quenching · Photoinhibition · Photoprotection · Photosystem II · Q_A · Q_B · Reaction centre quenching

Abbreviations

Cyt b_{559}	Cytochrome b_{559}
D1	Photosystem II reaction centre polypeptide
D2	Photosystem II reaction centre polypeptide
F_o	Minimum yield of chlorophyll fluorescence at open PSII centres in dark-adapted leaves
F_m	Maximum yield of fluorescence at closed PSII reaction centres in dark adapted leaves
F_v	Variable yield of fluorescence in dark adapted leaves
F_v/F_m	Maximum PSII photochemical efficiency in dark adapted leaves
LHCII	The major Chl a/b pigment-protein complex associated with PSII
NPQ	Non-photochemical quenching
OEC	Oxygen evolving complex
Pheo	Pheophytin
PI	Photoinhibition
PSI	Photosystem I
PSII	Photosystem II
PSIIα	Photosystem α-centres
PSIIβ	Photosystem β-centres
PsbS	PSII subunit and gene product of the *PsbS* gene
PQ	Plastoquinone
Q_A	Primary electron-accepting quinone in PSII reaction centres

A. G. Ivanov · N. P. A. Huner (✉)
Department of Biology and The Biotron, University of Western Ontario, 1151 Richmond Street N., London, ON, Canada N6A 5B7
e-mail: nhuner@uwo.ca

A. G. Ivanov · V. Hurry · G. Öquist
Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 901 87 Umea, Sweden

P. V. Sane
Jain Irrigation Systems Limited, Jain Hills, Jalgaon 425001, India

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REGULAR PAPER

Implications of alternative electron sinks in increased resistance of PSII and PSI photochemistry to high light stress in cold-acclimated *Arabidopsis thaliana*

A. G. Ivanov · D. Rosso · L. V. Savitch · P. Stachula · M. Rosembert · G. Oquist · V. Hurry · N. P. A. Hüner

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Abstract Exposure of control (non-hardened) *Arabidopsis* leaves to high light stress at 5 °C resulted in a decrease of both photosystem II (PSII) (45 %) and Photosystem I (PSI) (35 %) photochemical efficiencies compared to non-treated plants. In contrast, cold-acclimated (CA) leaves exhibited only 35 and 22 % decrease of PSII and PSI photochemistry, respectively, under the same conditions. This was accompanied by an accelerated rate of $P700^+$ re-reduction, indicating an up-regulation of PSI-dependent cyclic electron transport (CET). Interestingly, the expression of the NDH-H gene and the relative abundance of the Ndh-H polypeptide, representing the NDH-complex, decreased as a result of exposure to low temperatures. This indicates that the NDH-dependent CET pathway cannot be involved and the overall stimulation of CET in CA plants is due to up-regulation of the ferredoxin–plastoquinone reductase, antimycin A-sensitive CET pathway. The lower abundance of NDH complex also implies lower activity of the chlororespiratory pathway in CA plants, although the expression level and overall abundance of the other well-characterized component involved in chlororespiration, the plastid terminal oxidase (PTOX), was up-regulated at low temperatures. This suggests increased PTOX-mediated alternative electron flow to oxygen in plants exposed to low temperatures. Indeed, the estimated proportion of O_2-dependent linear electron transport not utilized in carbon assimilation and not directed to photorespiration was twofold higher in CA *Arabidopsis*. The possible involvement of alternative electron transport pathways in inducing greater resistance of both PSII and PSI to high light stress in CA plants is discussed.

Keywords Alternative electron flows · Cold acclimation · Photoprotection · Photosystem II · Photosystem I · PTOX

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A. G. Ivanov (✉) · D. Rosso · N. P. A. Hüner (✉)
Department of Biology, Western University,
1151 Richmond Street N., London, ON N6A 5B7, Canada
e-mail: aivanov@uwo.ca

N. P. A. Hüner
e-mail: nhuner@uwo.ca

L. V. Savitch · M. Rosembert
Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, ON K1A 0C6, Canada

P. Stachula · G. Oquist · V. Hurry
Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 901 87 Umeå, Sweden

Introduction

It is well established that exposure of leaves to low temperatures results in an imbalance between the capacity for harvesting light energy and the capacity to dissipate this energy through metabolic activity, resulting in excess PSII excitation pressure (Huner et al. 1996, 1998; Öquist and Huner 2003). The imbalance between the reducing equivalents produced in excess and the consumption capacity of photosynthesis can potentially result in generation of reactive oxygen species (ROS such as $^1O_2$ and O_2^-), which can lead to photoinhibition and photooxidative damage of photosystem II (PSII) (Powles 1984; Aro et al. 1993; Long et al. 1994) and photosystem I (PSI) (Sonoike et al. 1997; Ivanov et al. 1998; Scheller and Haldrup 2005). This potential for photoinhibition makes it necessary for the plant to develop mechanisms for photoprotection of the photosynthetic apparatus. In preparation for winter, temperate evergreen

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Iron stress responses in the cyanobacterium *Synechococcus* sp. PCC7942

Stefan Sandström[a], Alexander G Ivanov[b], Youn-Il Park[c], Gunnar Öquist[a] and Petter Gustafsson[a]

[a]*Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, S-90187 Umeå, Sweden*
[b]*Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada*
[c]*Department of Biology, Chungnam National University, Taejon 305-764, Korea*
Corresponding author, e-mail: stefan.sandstrom@plantphys.umu.se

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In the present study, we describe the sequential events by which the cyanobacterium *Synechococcus* sp. PCC 7942 adapts to iron deficiency. In doing so, we have tried to elucidate both short and long-term acclimation to low iron stress in order to understand how the photosynthetic apparatus adjusts to low iron conditions. Our results show that after an initial step, where CP43′ is induced and where ferredoxin is partly replaced by flavodoxin, the photosynthetic unit starts to undergo major rearrangements. All measured components of Photosystem I (PSI), PSII and cytochrome (Cyt) *f* decrease relative to chlorophyll (Chl) *a*. The photochemical efficiencies of the two photosystems also decline during this phase of acclimation. The well-known drop in phycobilisome content measured as phycocyanin (PC)/Chl was not due to an increased degradation, but rather to a decreased rate of synthesis. The largest effects of iron deficiency were observed on PSI, the most iron-rich structure of the photosynthetic apparatus. In the light of the recent discovery of an iron deficiency induced CP43′ ring around PSI a possible dual function of this protein as both an antenna and a quencher is discussed. We also describe the time course of a blue shift in the low temperature Chl emission peak around 715 nm, which originates in PSI. The shift might reflect the disassembly and/or degradation of PSI during iron deficiency and, as a consequence, PSI might under these conditions be found predominantly in a monomeric form. We suggest that the observed functional and compositional alterations represent cellular acclimation enabling growth and development under iron deficiency, and that growth ceases when the acclimation capacity is exhausted. However, the cells remain viable even after growth has ceased, since they resumed growth once iron was added back to the culture.

Introduction

Although iron is the fourth most abundant element on Earth, in oxic ecosystems and under physiological pHs, the biologically available Fe^{2+} is rapidly transformed into Fe^{3+} which in turn forms insoluble hydroxides. This creates a situation where iron is present but essentially unavailable to the biological system. Since the functional photosynthetic apparatus requires 22–23 iron atoms (Ferreira and Straus 1994) and iron is a key component of chromophore synthesis (Straus 1994), most obligate photoautotrophs, including cyanobacteria, are highly vulnerable to iron deficiency. To cope with the limited availability of iron in the surrounding environment, cyanobacteria develop iron scavenging systems, including synthesis of siderophores (Geider and La Roche 1994), as well as efficient ferric and ferrous iron transport systems over the plasma membrane (Katoh et al. 2001). In addition, the amounts of iron-containing proteins within the photosynthetic and respiratory electron transport chains are reduced (Sandman 1985). Cyanobacteria also undergo a number of morphological as well as physiological changes in response to iron limitation.

Earlier studies have reported that cells of *Synechococcus* sp., PCC 7942 grown under iron-deficient conditions, exhibit decreased PC and Chl content (Öquist 1974a, Guikema and Sherman 1983, Sandman 1985), a characteristic blue shift of the main red maximum

Abbreviations – A, absorbance; Chl, chlorophyll; Cyt, cytochrome; EDTA, ethylenediamine tetraacetic acid; F, Fluorescence; F_m, maximal chlorophyll fluorescence; F_o, instantaneous (dark) chlorophyll fluorescence; FR, far red light; F_v, variable fluorescence; MT, multiple turnover; PC, phycocyanin; PPFD, photosynthetic photon flux density; PSI, photosystem I; PSII, photosystem II; ST, single turnover.

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Low-temperature modulation of the redox properties of the acceptor side of photosystem II: photoprotection through reaction centre quenching of excess energy

Alexander G. Ivanov[a,b], Prafullachandra Sane[a,b], Vaughan Hurry[b,*], Marianna Król[a], Dimitry Sveshnikov[b], Norman P. A. Huner[a] and Gunnar Öquist[b]

[a]*Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7*
[b]*Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå S-901 87, Sweden*
**Corresponding author, e-mail: vaughan.hurry@plantphys.umu.se*

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Although it has been well established that acclimation to low growth temperatures is strongly correlated with an increased proportion of reduced Q_A in all photosynthetic groups, the precise mechanism controlling the redox state of Q_A and its physiological significance in developing cold tolerance in photoautotrophs has not been fully elucidated. Our recent thermoluminescence (TL) measurements of the acceptor site of PSII have revealed that short-term exposure of the cyanobacterium *Synechococcus* sp. PCC 7942 to cold stress, overwintering of Scots pine (*Pinus sylvestris* L.), and acclimation of *Arabidopsis* plants to low growth temperatures, all caused a substantial shift in the characteristic T_M of $S_2Q_B^-$ recombination to lower temperatures. These changes were accompanied by much lower overall TL emission, restricted electron transfer between Q_A and Q_B, and in *Arabidopsis* by a shift of the $S_2Q_A^-$-related peak to higher temperatures. The shifts in recombination temperatures are indicative of a lower activation energy for the $S_2Q_B^-$ redox pair and a higher activation energy for the $S_2Q_A^-$ redox pair. This results in an increase in the free-energy gap between $P680^+Q_A^-$ and $P680^+Pheo^-$ and a narrowing of the free energy gap between Q_A and Q_B electron acceptors. We propose that these effects result in an increased population of reduced Q_A (Q_A^-), facilitating non-radiative $P680^+Q_A^-$ radical pair recombination within the PSII reaction centre. The proposed reaction centre quenching could be an important protective mechanism in cyanobacteria in which antenna and zeaxanthin cycle-dependent quenching are not present. In herbaceous plants, the enhanced capacity for dissipation of excess light energy via PSII reaction centre quenching following cold acclimation may complement their capacity for increased utilization of absorbed light through CO_2 assimilation and carbon metabolism. During overwintering of evergreens, when photosynthesis is inhibited, PSII reaction centre quenching may complement non-photochemical quenching within the light-harvesting antenna when zeaxanthin cycle-dependent energy quenching is thermodynamically restricted by low temperatures. We suggest that PSII reaction centre quenching is a significant mechanism enabling cold-acclimated organisms to acquire increased resistance to high light.

Introduction

There is broad agreement that any environmentally induced imbalance between light energy supply and energy utilization through photosynthesis may cause photodamage to PSII and induce non-photochemical dissipation of excess light energy (non-photochemical quenching; NPQ) for photoprotection of PSII (Horton et al. 1996, Gilmore 1997). Radiationless dissipation of excess excitation energy in the chlorophyll pigment bed of LHCII, associated with the formation of the xanthophyll pigment zeaxanthin (Z), has been considered to

Abbreviations – CH, cold-acclimated plants; Cyt b_{559}, cytochrome b_{559}; F_o, minimum yield of chlorophyll fluorescence at open PSII centres in dark-adapted leaves; LHCII, light harvesting antenna of photosystem II; NH, non-hardened control plants; Pheo, pheophytin; PSI, PSII, photosystem I and photosystem II, respectively; PQ, plastoquinone; Q_A, primary electron-accepting quinone in PSII; Q_B, secondary electron-accepting quinone in PSII; NPQ, non-photochemical quenching coefficient; q_O, quenching coefficient for basal fluorescence; TL, thermoluminescence; T_M, temperature of maximum thermoluminescence emission.

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Photosynthetic Acclimation of the Filamentous Cyanobacterium, *Plectonema boryanum* UTEX 485, to Temperature and Light

Ewa Miśkiewicz [1], Alexander G. Ivanov [1], John P. Williams [2], Mobashsher U. Khan [2], Stefan Falk [3] and Norman P.A. Huner [1, 4]

[1] *Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada*
[2] *Department of Botany, University of Toronto, 25 Willcocks St, Toronto, Ontario M5S 3B2, Canada*
[3] *Department of Ekoteknik, Mid-Sweden University, Östersund, Sweden*

Photosynthetic acclimation to temperature and irradiance was studied in the filamentous, non-heterocystous cyanobacterium *Plectonema boryanum* UTEX 485. Growth rates of this cyanobacterium measured at ambient CO_2 were primarily influenced by temperature with minimal effects of irradiance. Both growth temperature and irradiance affected linolenic (18:3) and linoleic acid (18:2) levels in the four major lipid classes in an independent but additive manner. In contrast, photosynthetic acclimation was not due to either growth temperature or irradiance per se, but rather, due to the interaction of these environmental factors. *P. boryanum* grown at low temperature and moderate irradiance mimicked cells grown at high light. Compared to cells grown at either 29°C/150 μmol m^{-2} s^{-1} (29/150) or 15/10, *P. boryanum* grown at either15/150 or 29/750 exhibited: (1) reduced cellular levels of Chl *a* and phycobilisomes (PBS), and concomitantly higher content of an orange-red carotenoid, myxoxanthophyll; (2) higher light saturated rates (Pmax) when expressed on a Chl *a* basis but lower apparent quantum yields of oxygen evolution and (3) enhanced resistance to high light stress. *P. boryanum* grown at 15/150 regained normal blue-green pigmentation within 16 h after a temperature shift to 29°C at a constant irradiance of 150 μmol m^{-2} s^{-1}. DBMIB and KCN but not DCMU and atrazine partially inhibited the change in myxoxanthophyll/Chl *a* ratio following the shift from 15 to 29°C. We conclude that *P. boryanum* responds to either varying growth temperature or varying growth irradiance by adjusting the ability to absorb light through decreasing the cellular contents of Chl *a* and light-harvesting pigments and screening of excessive light by myxoxanthophyll predominantly localized in the cell wall/cell membrane to protect PSII from over-excitation. The possible role of redox sensing/signalling for photosynthetic acclimation of cyanobacteria to either temperature or irradiance is discussed.

Key words: Acclimation — Electron transport — Irradiance — Photoinhibition — *Plectonema boryanum* — Temperature.

Photosynthetic organisms must balance the processes of energy input and consumption to maintain maximal levels of energy conversion while avoiding over-reduction and subsequent damage of the photochemical apparatus (Huner et al. 1996, Durnford and Falkowski 1997, Huner et al. 1998). Any imbalance in energy budget, whereby the rate of light absorption exceeds the rate of energy utilization through metabolism and/or the rate of nonradiative dissipation of excess light, will be manifested as an increase in the redox poise of intersystem electron transport. Exposure of photoautotrophs to excess light energy results in an energy imbalance since the primary photochemical reactions occur on a much faster time scale than intersystem electron transport and metabolism (Huner et al. 1998). On the other hand, exposure to low temperature leads to the reduction in the rates of temperature-dependent metabolic processes and consequently the ability to utilise the light energy initially absorbed through temperature-independent photochemistry (Huner et al. 1998). As a consequence, photosynthetic acclimation to low temperature, in many respects, mimics photosynthetic acclimation to high light (Campbell et al. 1995, Escoubas et al. 1995, Maxwell et al. 1994, 1995a, b, Gray et al. 1996). Furthermore, green algae and plants appear to sense and respond to changes in the chloroplast redox poise rather than to absolute growth temperature and irradiance (Huner et al. 1996, 1998, Durnford and Falkowski 1997).

Photoautotrophs may exploit different mechanisms to counteract the imbalance in energy budget during exposure to fluctuations in environmental conditions. Green algae, *Chlorella vulgaris* and *Dunaliella salina*, adjust the structure and function of the photosynthetic apparatus to the conditions of excess excitation energy by reducing the size

Abbreviations: DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone; DGDG, digalactosyldiacylglycerol; FAME, fatty acid methyl esters; LHCII, PSII light-harvesting complex; Lhcb, Chl *a/b*-binding proteins of PSII; *Lhcb*, genes encoding Chl *a/b*-binding proteins of PSII; MGDG, monogalactosyldiacylglycerol; PBS, phycobilisome; PG, phosphatidylglycerol; Pmax, maximum light-saturated rate of photosynthesis; PMSF, phenylmethyl-sulfonyl fluoride; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; SQDG, sulfoquinovosyldiacylglycerol; TLC, thin-layer chromatography; 18:2, linoleic acid; 18:3, linolenic acid.

[4] Corresponding author: E-mail, nhuner@julian.uwo.ca; Fax, +1-519-661-3935.

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Temperature and Light Modulate the *trans*-Δ^3-Hexadecenoic Acid Content of Phosphatidylglycerol: Light-harvesting Complex II Organization and Non-photochemical Quenching

Gordon R. Gray [1,*], Alexander G. Ivanov [2], Marianna Król [2], John P. Williams [3], Mobashoher U. Kahn [3], Elizabeth G. Myscich [2] and Norman P. A. Huner [2]

[1] *Department of Plant Sciences, University of Saskatchewan, Saskatoon SK S7N 5A8, Canada*
[2] *Department of Biology and The Biotron, University of Western Ontario, London ON N6A 5B7, Canada*
[3] *Department of Botany, University of Toronto, Toronto ON M5S 1A1, Canada*

The interaction of light and temperature in the modulation of the *trans*-Δ^3-hexadecenoic acid (*trans*-16:1) content of phosphatidylglycerol (PG) in winter rye (*Secale cereale* L.) was assessed and related to the organization of light-harvesting complex II (LHCII). Increasing the growth irradiance from 50 to 800 μmol m^{-2} s^{-1} at 20°C resulted in a 1.8-fold increase in the *trans*-16:1 content in PG which favoured a greater preponderance of oligomeric LHCII, measured in vitro as the ratio of oligomer : monomer. Similar irradiance-dependent increases were observed during growth at 5°C; however, 1.4-fold lower *trans*-16:1 contents and lower LHCII oligomer : monomer ratios were observed compared with growth at 20°C and the same irradiance. These trends were also observed under natural field conditions. Thus, the accumulation of *trans*-16:1, as well as the organization of LHCII are modulated by both growth irradiance and growth temperature in an independent but additive manner. We also examined how changes in the supramolecular organization of LHCII affected the capacity for non-photochemical quenching (q_N) and photoprotection via antenna quenching (q_O). While q_O was positively correlated with q_N, there was no correlation with either LHCII organization or xanthophyll cycle activity under the steady-state growth conditions examined.

Keywords: Antenna quenching — Light-harvesting complex II — Non-photochemical quenching — Phosphatidylglycerol — Supramolecular organization — *trans*-Δ^3-hexadecenoic acid.

Abbreviations: A, antheraxanthin; EPS, epoxidation state; LHCII, light-harvesting complex II; Lhcb, Chl*a*/*b*-binding light-harvesting polypeptides associated with PSII; Lut, lutein; Neo, neoxanthin; PG, phosphatidylglycerol; Q_A, first stable electron acceptor of PSII; q_E, energy-dependent component of non-photochemical quenching; q_N, coefficient of non-photochemical quenching; q_O, quenching coefficient of basal fluorescence (antenna quenching); $1 - q_P$, relative reduction state of Q_A (PSII excitation pressure); *trans*-16:1, *trans*-Δ^3-hexadecenoic acid; V, violaxanthin; Z, zeaxanthin.

Introduction

In the natural environment, plants experience and must adjust to wide daily and seasonal fluctuations in temperature and light. The ability of plants to respond to changes in their environment is crucial in determining tolerance to stress and their habitat preference. When plants are exposed to excess light, a decrease in photosynthetic capacity, known as photoinhibition, frequently occurs due to an imbalance between the absorption of light energy and utilization through reductive carbon, nitrogen and sulfur metabolism. However, plants utilize different species-specific photoprotective strategies to minimize the potential for photoinhibition and subsequent photooxidative damage, comprised of both photochemical and non-photochemical mechanisms (Savitch et al. 2002, Öquist and Huner 2003). Growth and development of winter rye (*Secale cereale* L.) under conditions which result in elevated PSII excitation pressure result in a tolerance to photoinhibition (Gray et al. 1996). PSII excitation pressure is estimated by the Chl fluorescence parameter $1 - q_p$, and measures the relative reduction state of Q_A, the first stable quinone electron acceptor of PSII which, in turn, reflects the redox poise of the plastoquinone pool and intersystem electron transport chain (Dietz et al. 1985, Gray et al. 1996, Huner et al. 1998). Changes in either growth irradiance or growth temperature can modulate PSII excitation pressure in a similar manner. Lowering the temperature at constant irradiance lowers the capacity for light utilization which results in an increase in the reduction state of Q_A and hence an increase in $1 - q_P$. Alternatively, increasing the irradiance at constant temperature increases the rate of light absorption and produces an increase in reduction of Q_A (Maxwell et al. 1995, Gray et al. 1996, Huner et al. 1998). Furthermore, maximum photosynthetic capacity for oxygen evolution (P_{max} O_2), as well as non-photochemical quenching of Chl fluorescence (q_N), are positively and linearly correlated with $1 - q_p$ experienced during prevailing growth conditions in winter rye (Gray et al. 1996, Gray et al. 1997a).

Non-photochemical processes, collectively known as q_N, dissipate excess excitation energy in the antenna pigment bed of PSII and are considered to be the major PSII photoprotec-

* Corresponding author: E-mail, gr.gray@usask.ca; Fax, +1-306-966-5015.

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Digalactosyl-Diacylglycerol Deficiency Impairs the Capacity for Photosynthetic Intersystem Electron Transport and State Transitions in *Arabidopsis thaliana* Due to Photosystem I Acceptor-Side Limitations

Alexander G. Ivanov [1,2], Luke Hendrickson [2], Marianna Krol [1], Eva Selstam [2], Gunnar Öquist [2], Vaughan Hurry [2] and Norman P. A. Huner [1,*]

[1] *Department of Biology and The Biotron, University of Western Ontario, London, Ontario, Canada N6A 5B7*
[2] *Umea Plant Science Centre, Department of Plant Physiology, University of Umeå, Umeå S-901 87, Sweden*

Compared with wild type, the *dgd1* mutant of *Arabidopsis thaliana* exhibited a lower amount of PSI-related Chl–protein complexes and lower abundance of the PSI-associated polypeptides, PsaA, PsaB, PsaC, PsaL and PsaH, with no changes in the levels of Lhca1–4. Functionally, the *dgd1* mutant exhibited a significantly lower light-dependent, steady-state oxidation level of P700 ($P700^+$) in vivo, a higher intersystem electron pool size, restricted linear electron transport and a higher rate of reduction of $P700^+$ in the dark, indicating an increased capacity for PSI cyclic electron transfer compared with the wild type. Concomitantly, the *dgd1* mutant exhibited a higher sensitivity to and incomplete recovery of photoinhibition of PSI. Furthermore, *dgd1* exhibited a lower capacity to undergo state transitions compared with the wild type, which was associated with a higher reduction state of the plastoquinone (PQ) pool. We conclude that digalactosyl-diacylglycerol (DGDG) deficiency results in PSI acceptor-side limitations that alter the flux of electrons through the photosynthetic electron chain and impair the regulation of distribution of excitation energy between the photosystems. These results are discussed in terms of thylakoid membrane domain reorganization in response to DGDG deficiency in *A. thaliana*.

Keywords: *Arabidopsis dgd1* mutant — Chlorophyll fluorescence — Digalactosyl-diacylglycerol — Photoinhibition — P700 — State transitions.

Abbreviations: AL, actinic light; DGDG, digalactosyl-diaclyglycerol; F_o, instantaneous (minimal) chlorophyll fluorescence at open PSII centers in dark-adapted samples; $F_{o'}$, minimal fluorescence at open PSII centers immediately after illumination; F_m, maximal fluorescence at closed PSII centers; $F_{m'}$, maximal fluorescence at closed PSII centers under actinic light; F_s, steady-state fluorescence yield; F_v, variable fluorescence ($F_m - F_o$); $F_{v'}$, variable fluorescence under actinic light; $F_v/F_m = (F_m - F_o)/F_m$, the maximal photochemical efficiency of PSII; $F_{v'}/F_{m'} = (F_{m'} - F_{o'})/F_{m'}$, the efficiency of open PSII units under illumination; LHCII, the major Chl *a*/*b* pigment–protein complex associated with photosystem II; MGDG, monogalactosyl-diacylglycerol; P700, reaction center pigment of PSI; $P700^+$, oxidized form of the reaction center of PSI; PQ, plastoquinone; Q_A, primary electron-accepting quinone in PSII; Q_B, secondary electron-accepting quinone in PSII; q_N, non-photochemical quenching.

Introduction

It has been well established that membrane lipid composition is one of the important factors controlling the structure and function of thylakoid membranes via specific lipid–protein interactions and/or the dynamic properties of the lipid bilayer (Quinn and Williams 1983). The adaptation of photosynthetic membranes of a wide range of photoautotrophs (from cyanobacteria to higher plants) to various environmental conditions has been proposed to be accompanied, and in some cases sensed and even triggered by, changes in the bulk lipid composition and unsaturation of the thylakoid membrane lipids (Wada et al. 1990, Los and Murata 2002). The unique lipid composition of thylakoid membranes is dominated by two galactolipids, monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG), comprising approximately 50 and 20% of the total thylakoid acyl lipid content, respectively. The remaining lipid content is distributed between the negatively charged phosphatidylglycerol (PG), a sulfolipid sulfoquinovosyldiacylglycerol (SQDG) and some minor phospholipids (Quinn and Williams 1983, Webb and Green 1991).

Since the isolation and characterization of the *dgd1* mutant of *Arabidopsis thaliana*, lacking 96% of the wild-type level of DGDG (Dörman et al. 1995), the structure/functional role of DGDG in thylakoid membranes has been extensively studied. A linear relationship between PSII function and DGDG content of thylakoid membranes has been established (Härtel et al. 2001), the underlying structural/functional mechanisms of which have been extensively studied (Dörman et al. 1995, Härtel et al. 1997, Reifarth et al. 1997, Härtel et al. 1998, Steffen et al. 2005). Indeed, previous in vitro studies have demonstrated that removal of DGDG inhibits O_2 evolution in the

*Corresponding author: E-mail, nhuner@uwo.ca; Fax, +1-519-661-3935.

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Regulation of Energy Partitioning and Alternative Electron Transport Pathways During Cold Acclimation of Lodgepole Pine is Oxygen Dependent

Leonid V. Savitch[1,4,*], Alexander G. Ivanov[2,4], Marianna Krol[2], David P. Sprott[1], Gunnar Öquist[3] and Norman P. A. Huner[2]

[1]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, ON, Canada, K1A 0C6
[2]Department of Biology and The Biotron, The University of Western Ontario, London, ON, Canada, N6A 5B7
[3]Umeå Plant Science Center, Umeå University, Umeå S-901 87, Sweden
[4]These authors contributed equally to this work
*Corresponding author: E-mail, leonid.savitch@agr.gc.ca; Fax, +1 613-759-1701
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Second year needles of Lodgepole pine (*Pinus contorta* L.) were exposed for 6 weeks to either simulated control summer ['summer'; 25°C/250 photon flux denisty (PFD)], autumn ('autumn'; 15°C/250 PFD) or winter conditions ('winter'; 5°C/250 PFD). We report that the proportion of linear electron transport utilized in carbon assimilation (ETR_{CO2}) was 40% lower in both 'autumn' and 'winter' pine when compared with the 'summer' pine. In contrast, the proportion of excess photosynthetic linear electron transport (ETR_{excess}) not used for carbon assimilation within the total ETR_{Jf} increased by 30% in both 'autumn' and 'winter' pine. In 'autumn' pine acclimated to 15°C, the increased amounts of 'excess' electrons were directed equally to 21 kPa O_2-dependent and 2 kPa O_2-dependent alternative electron transport pathways and the fractions of excitation light energy utilized by PSII photochemistry (Φ_{PSII}), thermally dissipated through Φ_{NPQ} and dissipated by additional quenching mechanism(s) ($\Phi_{f,D}$) were similar to those in 'summer' pine. In contrast, in 'winter' needles acclimated to 5°C, 60% of photosynthetically generated 'excess' electrons were utilized through the 2 kPa O_2-dependent electron sink and only 15% by the photorespiratory (21 kPa O_2) electron pathway. Needles exposed to 'winter' conditions led to a 3-fold lower Φ_{PSII}, only a marginal increase in Φ_{NPQ} and a 2-fold higher $\Phi_{f,D}$, which was O_2 dependent compared with the 'summer' and 'autumn' pine. Our results demonstrate that the employment of a variety of alternative pathways for utilization of photosynthetically generated electrons by Lodgepole pine depends on the acclimation temperature. Furthermore, dissipation of excess light energy through constitutive non-photochemical quenching mechanisms is O_2 dependent.

Keywords: Chl fluorescence • Cold acclimation • Electron transport • Energy partitioning • Photochemistry • *Pinus contorta* L.

Abbreviations: CHB, cold-hard-band; ETR, electron transport rate; F_v/F_m, maximum photochemical efficiency of PSII with all reaction centers open; FWHM, full width at half maximum; LHCII, the major Chl *a*/*b* pigment–protein complex associated with PSII; NPQ, non-photochemical quenching; $p(CO_2)$, partial pressure of CO_2; $p(O_2)$, partial pressure of O_2; PFD, photon flux density; PTOX, plastid terminal oxidase; Q_A, primary electron-accepting quinone in PSII reaction centres; Q_B, secondary electron-accepting quinone in PSII reaction centers; qL, photochemical quenching.

Introduction

Exposure to low temperatures in combination with short days, conditions normally experienced during autumn in the northern temperate and boreal zones, induces cold and freezing tolerance and consequently dormancy in evergreen conifers, which is essential for their winter survival (Lindgren and Höllgren 1993, Colombo et al. 2001, Öquist et al. 2001, Öquist and Huner 2003). Cold acclimation in pine species triggered by decreasing day length and temperature involves the down-regulation of photosynthesis, induction of protective proteins and pigments, and adjustments of the antenna size and composition to favor decreased light absorption and increased dissipation of excess energy by non-photochemical quenching (NPQ) (Öquist and Huner 2003).

Cold acclimation followed by characteristic winter depression of photosynthesis in conifers (Pharis et al. 1970, Öquist and Huner 2003) is accompanied by seasonal changes in chloroplast ultrastructure (Perry and Baldwin 1966, Senser et al. 1975, Martin and Öquist 1979, Krol et al. 2002), re-localization of chloroplasts during winter (Tanaka 2007) as well as the distribution of the major Chl–protein complexes of PSII and PSI and the polypeptide composition of the thylakoid membranes (Öquist et al. 1978, Öquist and Strand 1986, Ottander et al. 1995,

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Cold Stress Effects on PSI Photochemistry in *Zea mays*: Differential Increase of FQR-Dependent Cyclic Electron Flow and Functional Implications

Leonid V. Savitch[1,4,*], Alexander G. Ivanov[2,3,4], Loreta Gudynaite-Savitch[2,5], Norman P. A. Huner[2,3] and John Simmonds[1]

[1]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre (ECORC), Central Experimental Farm, 960 Carling Ave., Ottawa, Ontario, Canada, K1A 0C6
[2]Department of Biology University of Western Ontario, London, Ontario, Canada N6A 5B7
[3]The Biotron Experimental Climate Change, University of Western Ontario, London, Ontario, Canada N6A 5B7
[4]These authors contributed equally to this work.
[5]Present address: Iogen Corporation, 310 Hunt Club Road East, Ottawa, ON, Canada K1V 1C1
*Corresponding author: E-mail, leonid.savitch@agr.gc.ca, Fax, +1-613-759-1701
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Cold-induced inhibition of CO_2 assimilation in maize (*Zea mays* L.) is associated with a persistent depression of the photochemical efficiency of PSII. However, very limited information is available on PSI photochemistry and PSI-dependent electron flow in cold-stressed maize. The extent of the absorbance change (ΔA_{820}) used for in vivo quantitative estimation of photooxidizable $P700^+$ indicated a 32% lower steady-state oxidation level of the PSI reaction center P700 ($P700^+$) in cold-stressed compared with control maize leaves. This was accompanied by a 2-fold faster re-reduction rate of $P700^+$ in the dark, indicating a higher capacity for cyclic electron flow (CEF) around PSI in cold-stressed maize leaves. Furthermore, the increased PSI-dependent CEF(s) was associated with a much higher stromal electron pool size and 56% lower capacity for state transitions compared with control plants. To examine NADP(H) dehydrogenase (NDH)- and ferredoxin:plastoquinone oxidoreductase (FQR)-dependent CEF in vivo, the post-illumination transient increase of F_o' was measured in the presence of electron transport inhibitors. The results indicate that under optimal growth conditions the relatively low CEF in the maize mesophyll cells is mostly due to the NDH-dependent pathway. However, the increased CEF in cold-stressed plants appears to originate from the up-regulated FQR pathway. The physiological role of PSI down-regulation, the increased capacity for CEF and the shift of preferred CEF mode in modulating the photosynthetic electron fluxes and distribution of excitation light energy in maize plants under cold stress conditions are discussed.

Keywords: Cold stress • Cyclic electron flow • P700 • PSI photochemistry • State transitions • *Zea mays* L.

Abbreviations: AL, actinic light; AntA, antimycin A; CEF, cyclic electron flow; ETR, electron transport rate; FNR, ferredoxin NADP+ oxidoreductase; FQR, ferredoxin:plastoquinone oxidoreductase; FR, far red; LHC, light-harvesting complex; MT, multiple turnover; NDH, NADP(H) dehydrogenase; NPQ, non-photochemical quenching; PQ, plastoquinone; Q_A, primary electron-accepting quinone in PSII reaction centers; ROS, reactive oxygen species; ST, single turnover; WWC, water–water cycle.

Introduction

The general physiological response of maize plants to low temperatures is an inhibition of photosynthetic CO_2 assimilation (Greer and Hardacre 1989, Kingston-Smith et al. 1997, Fryer et al. 1998, Foyer et al. 2002, Savitch et al. 2009). Cold-induced limitations on photosynthesis, i.e. a decrease in the photochemical use of absorbed light energy and the imbalance between the reducing equivalents produced in excess and the consumption capacity of photosynthesis, can induce the production of potentially dangerous reactive oxygen species (ROS) (Baker 1994). This may lead to photoinhibition of photosynthesis (Powles 1984, Baker 1994), and increased susceptibility to photoinhibition has been well established during exposure of photosynthetic organisms to low temperatures in combination with even moderate photon fluency rates (Öquist and Martin 1986, Greer et al. 1986, Greer 1990, Osmond 1994). PSII has been identified as the primary target of photoinhibition resulting in photooxidative damage of the D1 reaction center polypeptide of PSII (Krause 1988, Aro et al. 1993, Long et al. 1994). Indeed, decreased abundance and photodamage of the D1 protein have been reported in maize plants exposed to suboptimal temperatures (Nie et al. 1992, Baker 1994, Kingston-Smith et al. 1999, Savitch et al. 2009). It has been suggested that the persistent depression of photosynthetic efficiency in cold-acclimated

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Greening under High Light or Cold Temperature Affects the Level of Xanthophyll-Cycle Pigments, Early Light-Inducible Proteins, and Light-Harvesting Polypeptides in Wild-Type Barley and the *Chlorina f2* Mutant[1]

Marianna Król, Alexander G. Ivanov, Stefan Jansson, Klaus Kloppstech, and Norman P.A. Huner*

Department of Plant Sciences, The University of Western Ontario, London, Ontario, Canada N6A 5B7 (M.K., A.G.I., N.P.A.H.); Department of Plant Physiology, University of Umeå, S 901 87 Umeå, Sweden (S.J.); and Institut für Botanik, Universität Hannover, Herrnhäuser Strasse 2, Hannover 21 3000, Germany (K.K.)

Etiolated seedlings of wild type and the *chlorina f2* mutant of barley (*Hordeum vulgare*) were exposed to greening at either 5°C or 20°C and continuous illumination varying from 50 to 800 μmol m^{-2} s^{-1}. Exposure to either moderate temperature and high light or low temperature and moderate light inhibited chlorophyll *a* and *b* accumulation in the wild type and in the *f2* mutant. Continuous illumination under these greening conditions resulted in transient accumulations of zeaxanthin, concomitant transient decreases in violaxanthin, and fluctuations in the epoxidation state of the xanthophyll pool. Photoinhibition-induced xanthophyll-cycle activity was detectable after only 3 h of greening at 20°C and 250 μmol m^{-2} s^{-1}. Immunoblot analyses of the accumulation of the 14-kD early light-inducible protein but not the major (Lhcb2) or minor (Lhcb5) light-harvesting polypeptides demonstrated transient kinetics similar to those observed for zeaxanthin accumulation during greening at either 5°C or 20°C for both the wild type and the *f2* mutant. Furthermore, greening of the *f2* mutant at either 5°C or 20°C indicated that Lhcb2 is not essential for the regulation of the xanthophyll cycle in barley. These results are consistent with the thesis that early light-inducible proteins may bind zeaxanthin as well as other xanthophylls and dissipate excess light energy to protect the developing photosynthetic apparatus from excess excitation. We discuss the role of energy balance and photosystem II excitation pressure in the regulation of the xanthophyll cycle during chloroplast biogenesis in wild-type barley and the *f2* mutant.

Exposure of plants to fluctuations in irradiance in excess of that required for photosynthesis generally induces xanthophyll-cycle activity characterized by the reversible, light-dependent de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin. A strong correlation has been established between the nonphotochemical dissipation of excess light energy and zeaxanthin content, which protects PSII reaction centers from overexcitation (Demmig-Adams and Adams, 1992; Gilmore, 1997). Although the mechanism by which zeaxanthin is thought to dissipate excess energy nonphotochemically is still under debate (Horton et al., 1996; Owens, 1996), there is a general consensus that the antenna systems of PSI and PSII are the primary sites of nonphotochemical energy dissipation. Xanthophyll-cycle pigments are associated with the major and minor Lhcb polypeptides of LHCII and the Lhca polypeptides of the PSI light-harvesting complex (Bassi et al., 1993; Ruban et al., 1994).

Kloppstech and coworkers (Meyer and Kloppstech, 1984; Grimm and Kloppstech, 1987) were the first to report that ELIPs are transiently expressed during greening of etiolated barley (*Hordeum vulgare*) seedlings and mature leaves exposed to high-light stress. Furthermore, ELIPs and the PSII-S protein, which are thylakoid polypeptides induced under high-light stress and related to the Lhcb family of light-harvesting polypeptides, may also bind carotenoids to protect the photochemical apparatus from potential photooxidative damage upon exposure to excess light (Król et al., 1995; Adamska, 1997; Lindahl et al., 1997). In addition to its traditional role as a quencher of absorbed light energy when bound to antenna polypeptides, it has been proposed that unbound zeaxanthin and other carotenoids may also act to stabilize thylakoid membranes against potential peroxidative damage and heat stress (Havaux, 1998).

Angiosperms produce etiolated seedlings when exposed to prolonged darkness (Leech, 1984). Chloroplast biogenesis and assembly of the photosynthetic apparatus has generally been examined in monocots by exposure of etiolated

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* Corresponding author; e-mail nhuner@julian.uwo.ca; fax 1–519–661–3935.

Abbreviations: 5/50, low-temperature (5°C)/low-light (50 μmol m^{-2} s^{-1}) treatment; 5/250, low-temperature (5°C)/moderate-light (250 μmol m^{-2} s^{-1}) treatment; 20/250, moderate-temperature (20°C)/moderate-light (250 μmol m^{-2} s^{-1}) treatment; 20/800, moderate-temperature (20°C)/high-light (800 μmol m^{-2} s^{-1}) treatment; Chl, chlorophyll; ELIP, early light-inducible protein; F_m, maximum PSII fluorescence in the dark-adapted state; F_m', maximum PSII fluorescence in the light-adapted state; F_v, variable PSII fluorescence in the dark-adapted state; F_v', variable PSII fluorescence in the light-adapted state; F_v/F_m, maximum photochemical efficiency of PSII in the dark-adapted state; F_v'/F_m', photochemical efficiency of PSII during steady-state illumination; LHCII, PSII light-harvesting complex; qN, nonphotochemical quenching parameter; qP, photochemical quenching parameter.

Stoichiometry of the Photosynthetic Apparatus and Phycobilisome Structure of the Cyanobacterium *Plectonema boryanum* UTEX 485 Are Regulated by Both Light and Temperature[1]

Ewa Miskiewicz, Alexander G. Ivanov, and Norman P.A. Huner*

Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7

The role of growth temperature and growth irradiance on the regulation of the stoichiometry and function of the photosynthetic apparatus was examined in the cyanobacterium *Plectonema boryanum* UTEX 485 by comparing mid-log phase cultures grown at either 29°C/150 μmol m^{-2} s^{-1}, 29°C/750 μmol m^{-2} s^{-1}, 15°C/150 μmol m^{-2} s^{-1}, or 15°C/10 μmol m^{-2} s^{-1}. Cultures grown at 29°C/750 μmol m^{-2} s^{-1} were structurally and functionally similar to those grown at 15°C/150 μmol m^{-2} s^{-1}, whereas cultures grown at 29°C/150 μmol m^{-2} s^{-1} were structurally and functionally similar to those grown at 15°C/10 μmol m^{-2} s^{-1}. The stoichiometry of specific components of the photosynthetic apparatus, such as the ratio of photosystem (PS) I to PSII, phycobilisome size and the relative abundance of the cytochrome b_6/f complex, the plastoquinone pool size, and the NAD(P)H dehydrogenase complex were regulated by both growth temperature and growth irradiance in a similar manner. This indicates that temperature and irradiance may share a common sensing/signaling pathway to regulate the stoichiometry and function of the photosynthetic apparatus in *P. boryanum*. In contrast, the accumulation of neither the D1 polypeptide of PSII, the large subunit of Rubisco, nor the CF_1 α-subunit appeared to be regulated by the same mechanism. Measurements of P700 photooxidation in vivo in the presence and absence of inhibitors of photosynthetic electron transport coupled with immunoblots of the NAD(P)H dehydrogenase complex in cells grown at either 29°C/750 μmol m^{-2} s^{-1} or 15°C/150 μmol m^{-2} s^{-1} are consistent with an increased flow of respiratory electrons into the photosynthetic intersystem electron transport chain maintaining P700 in a reduced state relative to cells grown at either 29°C/150 μmol m^{-2} s^{-1} or 15°C/10 μmol m^{-2} s^{-1}. These results are discussed in terms of acclimation to excitation pressure imposed by either low growth temperature or high growth irradiance.

Cyanobacteria are a large and diverse group of prokaryotes performing oxygenic photosynthesis in a manner similar to green algae and plants. The cyanobacterial photosynthetic apparatus consists of five multiprotein complexes. PSII, cytochrome b_6f, PSI, and ATP synthase are common to both cyanobacteria and plants. However, the fifth complex, a light-harvesting antenna of PSII, is functionally but not structurally homologous (Gantt, 1994). In cyanobacteria, light harvesting is mediated by phycobilisomes (PBSs), complex protein structures located on the cytoplasmic surface of the thylakoid membranes. The major components of the PBS are the biliproteins, allophycocyanin (AP), phycocyanin (PC), phycoerythrin, and phycoerythrocyanin, with covalently attached bilin chromophores. Different colorless linker polypeptides are specifically associated with each type of phycobiliprotein and function to stabilize the PBS and optimize their absorbance and energy transfer characteristics. The PBSs are composed of two structural domains: an AP core that is in direct contact with the thylakoid membrane and generally six rods of stacked PC and, in some strains, phycoerythrin or phycoerythrocyanin hexamers radiating from the core (Sidler, 1994).

Another important distinction between cyanobacteria and chloroplasts is that in cyanobacteria, both respiratory and photosynthetic electron transport chains function within thylakoid membranes, where they share electron transport components such as the plastoquinone (PQ) pool and cytochrome b_6f complex (Scherer, 1990; Schmetterer, 1994; Cooley et al., 2000; Cooley and Vermaas, 2001). Thus, electron fluxes in the intersystem chain may be affected by the electron supply from PSII, NAD(P)H dehydrogenase (Ndh)-, and succinate dehydrogenase-mediated electron transport pathway from respiratory donors and cyclic electron pathway around PSI as well as the electron consumption by cytochrome oxidase (Cooley et al., 2000; Cooley and Vermaas, 2001).

The composition of cyanobacterial photosynthetic apparatus is regulated in response to environmental factors such as light, temperature, and nutrient availability. Growth of cyanobacteria at high irradiance induces changes in the abundance of light-harvesting antennae (Raps et al., 1985; de Lorimier et al., 1992; Reuter and Muller, 1993; Garnier et al., 1994; Samson

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* Corresponding author; e-mail nhuner@uwo.ca; fax 519–661–3935.

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Changes in the Redox Potential of Primary and Secondary Electron-Accepting Quinones in Photosystem II Confer Increased Resistance to Photoinhibition in Low-Temperature-Acclimated Arabidopsis[1]

Prafullachandra Vishnu Sane, Alexander G. Ivanov, Vaughan Hurry, Norman P.A. Huner, and Gunnar Öquist*

Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå S–901 87, Sweden (P.V.S., V.H., G.O.); and Biology Department, University of Western Ontario, London, Ontario, Canada N6A 5B7 (A.G.I., N.P.A.H.)

Exposure of control (non-hardened) Arabidopsis leaves for 2 h at high irradiance at 5°C resulted in a 55% decrease in photosystem II (PSII) photochemical efficiency as indicated by F_v/F_m. In contrast, cold-acclimated leaves exposed to the same conditions showed only a 22% decrease in F_v/F_m. Thermoluminescence was used to assess the possible role(s) of PSII recombination events in this differential resistance to photoinhibition. Thermoluminescence measurements of PSII revealed that $S_2Q_A^-$ recombination was shifted to higher temperatures, whereas the characteristic temperature of the $S_2Q_B^-$ recombination was shifted to lower temperatures in cold-acclimated plants. These shifts in recombination temperatures indicate higher activation energy for the $S_2Q_A^-$ redox pair and lower activation energy for the $S_2Q_B^-$ redox pair. This results in an increase in the free-energy gap between $P680^+Q_A^-$ and $P680^+Pheo^-$ and a narrowing of the free energy gap between primary and secondary electron-accepting quinones in PSII electron acceptors. We propose that these effects result in an increased population of reduced primary electron-accepting quinone in PSII, facilitating non-radiative $P680^+Q_A^-$ radical pair recombination. Enhanced reaction center quenching was confirmed using in vivo chlorophyll fluorescence-quenching analysis. The enhanced dissipation of excess light energy within the reaction center of PSII, in part, accounts for the observed increase in resistance to high-light stress in cold-acclimated Arabidopsis plants.

It has been shown previously in winter cereals (Öquist and Huner, 1993) and Arabidopsis (Strand et al., 1997, 1999) that cold acclimation results in an increased capacity for photosynthesis at suboptimal temperatures. This recovery in photosynthetic capacity is closely associated with the posttranslational activation and the selective increase in the expression of enzymes involved in Suc synthesis, with changes in expression and activity of Calvin cycle enzymes (Strand et al., 1997, 1999, 2003; Hurry et al., 2000; Stitt and Hurry, 2002), and with changes in the lipid composition and the content of unsaturated fatty acids of chloroplast membranes (Raison et al., 1982; Hugly and Somerville, 1992; Moon et al., 1995; Routaboul et al., 2000). These changes in photosynthetic capacity and in chloroplast membrane composition protect the photosynthetic apparatus against photoinhibition at low temperatures by allowing increased turnover of the photosynthetic electron transport chain (Hurry et al., 1993, 1995; Huner et al., 1998).

However, results obtained with Scots pine (*Pinus sylvestris*) indicate that cold acclimation can increase the level of photosystem II (PSII) resistance to excessive light directly without any increase in photosynthetic capacity (Krivosheeva et al., 1996). The acquisition of increased tolerance to photoinhibition in cold-acclimated plants has also been ascribed to growth and development under high PSII excitation pressure, i.e. growth conditions that result in a higher reduction state of primary electron-accepting quinone in PSII (Q_A) during steady-state growth at suboptimal temperatures due to an increased reduction of the intersystem electron transport chain (Maxwell et al., 1995; Gray et al., 1996; Huner et al., 1998). It is established that exposure to low nonfreezing temperatures results in an increased PSII excitation pressure, measured as $1 - q_P = \{(Q_A)_{red}/[(Q_A)_{red} + (Q_A)_{ox}]\}$ due to the temperature-dependent decrease in the capacity to use photosynthetic reductants through metabolism. This is a fundamental feature of all taxonomic groups of photosynthetic organisms (Öquist and Huner, 1993; Öquist et al., 1993; Maxwell et al., 1995; Gray et al., 1996; Huner et al., 1998). Thus, although photosynthetic acclimation to low temperature may be regulated, in part, by cellular metabolic

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* Corresponding author; e-mail gunnar.oquist@plantphys.umu.se; fax 46–90–7866676.

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Iron Deficiency in Cyanobacteria Causes Monomerization of Photosystem I Trimers and Reduces the Capacity for State Transitions and the Effective Absorption Cross Section of Photosystem I in Vivo[1]

Alexander G. Ivanov, Marianna Krol, Dmitry Sveshnikov, Eva Selstam, Stefan Sandström, Maryam Koochek, Youn-Il Park, Sergej Vasil'ev, Doug Bruce, Gunnar Öquist, and Norman P.A. Huner*

Department of Biology and The Biotron, University of Western Ontario, London, Ontario, Canada N6A 5B7 (A.G.I., M. Krol, N.P.A.H.); Department of Plant Physiology, University of Umeå, Umea S–901 87, Sweden (A.G.I., D.S., E.S., S.S., G.O.); Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1 (M. Koochek, S.V., D.B.); and Department of Biology, Chungnam National University, Daejon 305–764, Korea (Y.-I.P.)

The induction of the isiA (CP43′) protein in iron-stressed cyanobacteria is accompanied by the formation of a ring of 18 CP43′ proteins around the photosystem I (PSI) trimer and is thought to increase the absorption cross section of PSI within the CP43′-PSI supercomplex. In contrast to these in vitro studies, our in vivo measurements failed to demonstrate any increase of the PSI absorption cross section in two strains (*Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803) of iron-stressed cells. We report that iron-stressed cells exhibited a reduced capacity for state transitions and limited dark reduction of the plastoquinone pool, which accounts for the increase in PSII-related 685 nm chlorophyll fluorescence under iron deficiency. This was accompanied by lower abundance of the NADP-dehydrogenase complex and the PSI-associated subunit PsaL, as well as a reduced amount of phosphatidylglycerol. Nondenaturating polyacrylamide gel electrophoresis separation of the chlorophyll-protein complexes indicated that the monomeric form of PSI is favored over the trimeric form of PSI under iron stress. Thus, we demonstrate that the induction of CP43′ does not increase the PSI functional absorption cross section of whole cells in vivo, but rather, induces monomerization of PSI trimers and reduces the capacity for state transitions. We discuss the role of CP43′ as an effective energy quencher to photoprotect PSII and PSI under unfavorable environmental conditions in cyanobacteria in vivo.

Among various structural (Sherman and Sherman, 1983) and functional responses of cyanobacterial cells to growth under iron-limited conditions (Öquist, 1974a, 1974b; Guikema and Sherman, 1983; Ivanov et al., 2000; Sandström et al., 2001, 2002; Michel and Pistorius, 2004), one of the most specific is the appearance of a chlorophyll (Chl)-protein complex associated with the *isiA* gene product CP43′ (Pakrasi et al., 1985a, 1985b; Riethman and Sherman, 1988; Burnap et al., 1993). Since CP43′ possesses high homology to the Chl *a*-binding protein CP43 of PSII core (Burnap et al., 1993; Falk et al., 1995), the increase of the low temperature (77 K) PSII-related Chl fluorescence peak at 685 nm in iron-stressed cells was initially linked to the induction of the CP43′ polypeptide (Burnap et al., 1993; Falk et al., 1995; Park et al., 1999). It was suggested that CP43′ could replace CP43, thus acting as an alternative antenna complex for PSII (Pakrasi et al., 1985b) or as a quencher with the ability to protect PSII from photoinhibitory damage during iron starvation (Park et al., 1999; Sandström et al., 2001; Cadoret et al., 2004; Ihalainen et al., 2005). The induction of the IsiA protein under strong light even in the presence of iron confirmed its photoprotective role (Havaux et al., 2005). Furthermore, the expression of *isiA* after salt stress (Hagemann et al., 1999), heat stress (Fulda and Hagemann, 1995), oxidative stress (Jeanjean et al., 2003; Yousef et al., 2003), and in a Cytc_6-deficient mutant of *Synechocystis* sp. PCC 6803 (Ardelean et al., 2002) indicates that the induction of *isiA/B* expression is not restricted to iron stress, but rather should be considered as a general stress response.

Earlier studies reported major alterations in the composition of pigment-protein complexes in cyanobacteria under iron stress (Guikema and Sherman, 1983; Pakrasi et al., 1985a, 1985b) and suggested that CP43′ could be associated not only with PSII but also with the PSI complex (Pakrasi et al., 1985a, 1985b). More recently, the induction of CP43′ under iron stress was

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* Corresponding author; e-mail nhuner@uwo.ca; fax 519–661–3935.

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IMMUTANS Does Not Act as a Stress-Induced Safety Valve in the Protection of the Photosynthetic Apparatus of Arabidopsis during Steady-State Photosynthesis[1]

Dominic Rosso, Alexander G. Ivanov, Aigen Fu, Jane Geisler-Lee, Luke Hendrickson, Matt Geisler, Gregory Stewart, Marianna Krol, Vaughan Hurry, Steven R. Rodermel, Denis P. Maxwell, and Norman P.A. Hüner*

Department of Biology and The Biotron, University of Western Ontario, London, Ontario, Canada, N6A 5B7 (D.R., A.G.I., J.G.-L., L.H., M.G., G.S., M.K., D.P.M., N.P.A.H.); Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa 50011 (A.F., S.R.R.); and Umea Plant Science Centre, Department of Plant Physiology, Umea University, Umea S–901 87, Sweden (L.H., V.H.)

IMMUTANS (*IM*) encodes a thylakoid membrane protein that has been hypothesized to act as a terminal oxidase that couples the reduction of O_2 to the oxidation of the plastoquinone (PQ) pool of the photosynthetic electron transport chain. Because *IM* shares sequence similarity to the stress-induced mitochondrial alternative oxidase (AOX), it has been suggested that the protein encoded by *IM* acts as a safety valve during the generation of excess photosynthetically generated electrons. We combined in vivo chlorophyll fluorescence quenching analyses with measurements of the redox state of P_{700} to assess the capacity of IM to compete with photosystem I for intersystem electrons during steady-state photosynthesis in Arabidopsis (*Arabidopsis thaliana*). Comparisons were made between wild-type plants, *im* mutant plants, as well as transgenics in which IM protein levels had been overexpressed six (OE-6 ×) and 16 (OE-16 ×) times. Immunoblots indicated that IM abundance was the only major variant that we could detect between these genotypes. Overexpression of *IM* did not result in increased capacity to keep the PQ pool oxidized compared to either the wild type or *im* grown under control conditions (25°C and photosynthetic photon flux density of 150 μmol photons m^{-2} s^{-1}). Similar results were observed either after 3-d cold stress at 5°C or after full-leaf expansion at 5°C and photosynthetic photon flux density of 150 μmol photons m^{-2} s^{-1}. Furthermore, IM abundance did not enhance protection of either photosystem II or photosystem I from photoinhibition at either 25°C or 5°C. Our in vivo data indicate that modulation of *IM* expression and polypeptide accumulation does not alter the flux of intersystem electrons to P_{700}^{+} during steady-state photosynthesis and does not provide any significant photoprotection. In contrast to *AOX1a*, meta-analyses of published Arabidopsis microarray data indicated that *IM* expression exhibited minimal modulation in response to myriad abiotic stresses, which is consistent with our functional data. However, *IM* exhibited significant modulation in response to development in concert with changes in *AOX1a* expression. Thus, neither our functional analyses of the *IM* knockout and overexpression lines nor meta-analyses of gene expression support the model that IM acts as a safety valve to regulate the redox state of the PQ pool during stress and acclimation. Rather, *IM* appears to be strongly regulated by developmental stage of Arabidopsis.

Plants with a variegated phenotype display distinct color variation in their vegetative organs, the most common of which being leaves with distinct green/white sectoring (Kirk and Tilney-Bassett, 1978; Rodermel, 2002). Whereas cells in the green sectors contain essentially normal chloroplasts, cells in the white sectors have plastids that are deficient in chlorophyll (Chl) and/or carotenoids (Rodermel, 2001, 2002). The variegated phenotype is most often the result of mutations to distinct genes of either the nuclear or organellar genomes (Tilney-Bassett, 1975) and include the maize (*Zea mays*) *nonchromosomal stripe* (*ncs*) and *iojap*, as well as Arabidopsis (*Arabidopsis thaliana*) mutants, *chloroplast mutator* (*chm*), *pale cress* (*pac*), *var1* and *var2*, *cab underexpressed* (*cue1*), and *immutans* (*im*; for review, see Rodermel, 2002).

The *im* mutant was isolated and initially characterized nearly 40 years ago by Rédei and coworkers, who showed that *im* is the result of a recessive mutation to a nuclear gene and that the resulting variegated phenotype is exacerbated by exposure to elevated temperatures and high light intensities (Rédei, 1963, 1975; Röbbelen, 1968; Wetzel et al., 1994). More recently, Wetzel et al. (1994) found that whereas the green sectors contain the normal allotment of Chls and colored carotenoids, the white sectors showed an accumulation of the colorless carotenoid phytoene, the precursor of the major colored carotenoids. The white sectoring seen when plants are grown under moderate to high irradiance is thus presumed to be the result of the photooxidation of

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* Corresponding author; e-mail nhuner@uwo.ca; fax 519–661–3935.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Norman P.A. Hüner (nhuner@uwo.ca).

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ORIGINAL ARTICLE

**Leonid V. Savitch · Johan Barker-Åstrom
Alexander G. Ivanov · Vaughan Hurry · Gunnar Öquist
Norman P.A. Huner · Per Gardeström**

Cold acclimation of *Arabidopsis thaliana* results in incomplete recovery of photosynthetic capacity, associated with an increased reduction of the chloroplast stroma

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Abstract The effects of short-term cold stress and long-term cold acclimation on the light reactions of photosynthesis were examined in vivo to assess their contributions to photosynthetic acclimation to low temperature in *Arabidopsis thaliana* (L.) Heynh.. All photosynthetic measurements were made at the temperature of exposure: 23 °C for non-acclimated plants and 5 °C for cold-stressed and cold-acclimated plants. Three-day cold-stress treatments at 5 °C inhibited light-saturated rates of CO_2 assimilation and O_2 evolution by approximately 75%. The 3-day exposure to 5 °C also increased the proportion of reduced Q_A by 50%, decreased the yield of PSII electron transport by 65% and decreased PSI activity by 31%. In contrast, long-term cold acclimation resulted in a strong but incomplete recovery of light-saturated photosynthesis at 5 °C. The rates of light-saturated CO_2 and O_2 gas exchange and the in vivo yield of PSII activity under light-saturating conditions were only 35–40% lower, and the relative redox state of Q_A only 20% lower, at 5 °C after cold acclimation than in controls at 23 °C. PSI activity showed full recovery during long-term cold acclimation. Neither short-term cold stress nor long-term cold acclimation of *Arabidopsis* was associated with a limitation in ATP, and both treatments resulted in an increase in the ATP/NADPH ratio. This increase in ATP/NADPH was associated with an inhibition of PSI cyclic electron transport but there was no apparent change in the Mehler reaction activity in either cold-stressed or cold-acclimated leaves. Cold acclimation also resulted in an increase in the reduction state of the stroma, as indicated by an increased total activity and activation state of NADP-dependent malate dehydrogenase, and increased light-dependent activities of the major regulatory enzymes of the oxidative pentose-phosphate pathway. We suggest that the photosynthetic capacity during cold stress as well as cold acclimation is altered by limitations at the level of consumption of reducing power in carbon metabolism.

Keywords *Arabidopsis* (cold stress) · Cold acclimation · Photosystem I (II) · Reduction state of stroma

Abbreviations Chl: chlorophyll · Fru6P: fructose 6-phosphate · Glc6P: glucose 6-phosphate · Glc1P: glucose 1-phosphate · MDH: malate dehydrogenase · PSI, PSII: photosystem I, II · PQ(A): plastoquinone (A) · P700: the reaction center of PSI · $P700^+$: the oxidized form of the reaction center of PSI · $p(CO_2)$: partial pressure of CO_2 · $p(O_2)$: partial pressure of O_2 · qN: non-photochemical quenching · qP: photochemical quenching · PGA: glycerate-3-phosphate · Q_A: the first stable primary quinone electron acceptor of PSII reaction centers · θ_{PSII}: quantum yield of PSII · $t_{1/2}$: half-time for completion of an event · TP: triose phosphate

J. Barker-Åstrom · V. Hurry · G. Öquist · P. Gardeström (✉)
Umeå Plant Science Centre,
Department of Plant Physiology,
Umeå University, 901 87 Umeå, Sweden
E-mail: per.gardestrom@plantphys.umu.se
Fax: +46-90-7866676

L.V. Savitch[1] · A.G. Ivanov · N.P.A. Huner
Department of Plant Sciences,
The University of Western Ontario,
London N6A 5B7, Ontario, Canada

Present address:
[1]Agriculture and Agri-Food Canada
Eastern Cereal and Oilseed Research Centre (ECORC),
Ottawa K1A 0C6, Ontario, Canada

Introduction

Photosynthetic acclimation to low temperature (5 °C) has been studied extensively in a range of hardy and non-hardy cereals (Huner et al. 1993; Hurry et al. 1994, 1995; Gray et al. 1996; Savitch et al. 1997). In the short-term (hours to days), low-temperature stress results in an

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ORIGINAL ARTICLE

Rachael M. Morgan-Kiss · Alexander G. Ivanov
Norman P.A. Huner

The Antarctic psychrophile, *Chlamydomonas subcaudata*, is deficient in state I–state II transitions

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Abstract State I–State II transitions were monitored in vivo and in vitro in the Antarctic, psychrophillic, green alga, *Chlamydomonas subcaudata*, as changes in the low-temperature (77 K) chlorophyll fluorescence emission maxima at 722 nm (F_{722}) relative to 699 nm (F_{699}). As expected, the control mesophillic species, *Chlamydomonas reinhardtii*, was able to modulate the light energy distribution between photosystem II and photosystem I in response to exposure to four different conditions: (i) dark/anaerobic conditions, (ii) a change in Mg^{2+} concentration, (iii) red light, and (iv) increased incubation temperature. This was correlated with the ability to phosphorylate both of its major light-harvesting polypeptides. In contrast, exposure of *C. subcaudata* to the same four conditions induced minimum alterations in the 77 K fluorescence emission spectra, which was correlated with the ability to phosphorylate only one of its major light-harvesting polypeptides. Thus, *C. subcaudata* appears to be deficient in the ability to undergo a State I–State II transition. Functionally, this is associated with alterations in the apparent redox status of the intersystem electron transport chain and with higher rates of photosystem I cyclic electron transport in the psychrophile than in the mesophile, based on in vivo P_{700} measurements. Structurally, this deficiency is associated with reduced levels of Psa A/B relative to D1, the absence of specific photosystem I light-harvesting polypeptides [R.M. Morgan et al. (1998) Photosynth Res 56:303–314] and a cytochrome b_6/f complex that exhibits a form of cytochrome *f* that is approximately 7 kDa smaller than that observed in *C. reinhardtii*. We conclude that the Antarctic psychrophile, *C. subcaudata*, is an example of a natural variant deficient in State I–State II transitions.

Keywords Anaerobiosis · Cations · *Chlamydomonas* (state transitions) · Light quality · State transitions · Temperature

Abbreviations ΔA_{820}: change in absorbance at 820 nm · Chl: chlorophyll · Cyt *f*: cytochrome *f* · D1: photosystem II reaction-center polypeptide · DCMU: 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea · DBMIB: 2,5-dibromo-3-ethyl-6-isopropyl-*p*-benzoquinone · $F_{688,\ 699,\ 700,\ 715,\ 722}$: 77 K fluorescence emission maxima at the respective wavelengths · FR: far-red light · LHCI: light-harvesting complex I · LHCII: light-harvesting complex II · MT: multiple turnover · P_{700}: photosystem I reaction center · PMSF: phenylmethylsulfonyl fluoride · PQ: plastoquinone · PsaA/PsaB: heterodimer reaction-center protein complex of PSI · ST: single turnover · MBZ: 3,3',5,5'-tetramethylbenzidine

Introduction

Chlamydomonas subcaudata was isolated by Priscu and co-workers from the unique aquatic habitat of a perennially ice-covered lake (Lake Bonney) located in the Taylor Dry Valleys, South Victoria Land, Antarctica (Neale and Priscu 1995). The environment of the Dry Valley lakes provides an extremely stable growth regime year round for the microalgae that inhabit them. *C. subcaudata* was identified in the lowest trophic level of the water column, about 17 m below the ice. Temperatures range from 0 to –2 °C with an irradiance of less than 50 μmol photons m^{-2} s^{-1} in the blue-green spectral range (Lizotte and Priscu 1992b). Thus, unlike most phytoplankton isolated from aquatic habitats, *C. subcaudata* appears to be adapted to annual growth under a unique but fairly constant regime of temperature, irradiance and spectral quality (Neale and Priscu 1995).

R.M. Morgan-Kiss · A.G. Ivanov · N.P.A. Huner (✉)
The Department of Plant Sciences,
The University of Western Ontario,
1151 Richmond Street N., London,
Ontario, N6A 5B7, Canada
E-mail: nhuner@uwo.ca
Fax: +519 661-3935

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ORIGINAL ARTICLE

A.G. Ivanov · P.V. Sane · Y. Zeinalov · I. Simidjiev
N.P.A. Huner · G. Öquist

Seasonal responses of photosynthetic electron transport in Scots pine (*Pinus sylvestris* L.) studied by thermoluminescence

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Abstract The potential of photosynthesis to recover from winter stress was studied by following the thermoluminescence (TL) and chlorophyll fluorescence changes of winter pine needles during the exposure to room temperature (20 °C) and an irradiance of 100 μmol m^{-2} s^{-1}. TL measurements of photosystem II (PSII) revealed that the $S_2Q_B^-$ charge recombinations (the B-band) were shifted to lower temperatures in winter pine needles, while the $S_2Q_A^-$ recombinations (the Q-band) remained close to 0 °C. This was accompanied by a drastically reduced (65%) PSII photochemical efficiency measured as F_v/F_m, and a 20-fold faster rate of the fluorescence transient from F_o to F_m as compared to summer pine. A strong positive correlation between the increase in the photochemical efficiency of PSII and the increase in the relative contribution of the B-band was found during the time course of the recovery process. The seasonal dynamics of TL in Scots pine needles studied under field conditions revealed that between November and April, the contribution of the Q- and B-bands to the overall TL emission was very low (less than 5%). During spring, the relative contribution of the Q- and B-bands, corresponding to charge recombination events between the acceptor and donor sides of PSII, rapidly increased, reaching maximal values in late July. A sharp decline of the B-band was observed in late summer, followed by a gradual decrease, reaching minimal values in November. Possible mechanisms of the seasonally induced changes in the redox properties of $S_2/S_3Q_B^-$ recombinations are discussed. It is proposed that the lowered redox potential of Q_B in winter needles increases the population of Q_A^-, thus enhancing the probability for non-radiative $P680^+Q_A^-$ recombination. This is suggested to enhance the radiationless dissipation of excess light within the PSII reaction center during cold acclimation and during cold winter periods.

Keywords Electron transport · Photosystem II · *Pinus* (cold acclimation) · Recovery of photosynthesis · Thermoluminescence · Winter stress

Abbreviations DCMU: 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea · F_o: minimum yield of chlorophyll fluorescence at open PSII centers in dark-adapted needles · P680: reaction-center pigment of PSII · $P680^+$: oxidized form of the reaction center of PSII · PSI, PSII: photosystem I and photosystem II, respectively · PPFD: photosynthetic photon flux density · PQ: plastoquinone · Q_A: primary electron-accepting quinone in PSII · Q_B: secondary electron-accepting quinone in PSII · SP: summer pine · TL: thermoluminescence · T_M: temperature of maximum thermoluminescence emission · WP: winter pine

A.G. Ivanov · P.V. Sane · I. Simidjiev · G. Öquist (✉)
Umeå Plant Science Center, Department of Plant Physiology, University of Umeå, Umeå 901 87, Sweden
E-mail: gunnar.oquist@plantphys.umu.se
Fax: +46-90-7866676

A.G. Ivanov · N.P.A. Huner
Department of Plant Sciences, University of Western Ontario, London, Ontario, N6A 5B7, Canada

Y. Zeinalov
Institute of Plant Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

I. Simidjiev
Department of Molecular Biology, University of Geneva, 1211 Geneva 4, Switzerland

Introduction

The characteristic winter depression of photosynthesis in conifers (Pharis et al. 1970) is accompanied by major changes in chloroplast ultrastructure (Senser et al. 1975; Martin and Öquist 1979) as well as in the lipid (Öquist 1982) and protein composition of the thylakoid membranes (Öquist et al. 1978; Ottander et al. 1995; Vogg et al. 1998). The seasonal changes in the structure and composition of the photosynthetic

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ORIGINAL ARTICLE

**Alexander G. Ivanov · Marianna Krol
Dimitri Sveshnikov · Gunilla Malmberg
Per Gardeström · Vaughan Hurry · Gunnar Öquist
Norman P. A. Huner**

Characterization of the photosynthetic apparatus in cortical bark chlorenchyma of Scots pine

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Abstract Winter-induced inhibition of photosynthesis in Scots pine (*Pinus sylvestris* L.) needles is accompanied by a 65% reduction of the maximum photochemical efficiency of photosystem II (PSII), measured as F_v/F_m, but relatively stable photosystem I (PSI) activity. In contrast, the photochemical efficiency of PSII in bark chlorenchyma of Scots pine twigs was shown to be well preserved, while PSI capacity was severely decreased. Low-temperature (77 K) chlorophyll fluorescence measurements also revealed lower relative fluorescence intensity emitted from PSI in bark chlorenchyma compared to needles regardless of the growing season. Nondenaturating SDS-PAGE analysis of the chlorophyll–protein complexes also revealed much lower abundance of LHCI and the CPI band related to light harvesting and the core complex of PSI, respectively, in bark chlorenchyma. These changes were associated with a 38% reduction in the total amount of chlorophyll in the bark chlorenchyma relative to winter needles, but the Chl *a*/*b* ratio and carotenoid composition were similar in the two tissues. As distinct from winter pine needles exhibiting ATP/ADP ratio of 11.3, the total adenylate content in winter bark chlorenchyma was 2.5-fold higher and the estimated ATP/ADP ratio was 20.7. The photochemical efficiency of PSII in needles attached to the twig recovered significantly faster (28–30 h) then in detached needles. Fluorescence quenching analysis revealed a high reduction state of Q_A and the PQ-pool in the green bark tissue. The role of bark chlorenchyma and its photochemical performance during the recovery of photosynthesis from winter stress in Scots pine is discussed.

Keywords Bark chlorenchyma · Chlorophyll fluorescence · Energy partitioning P700 · *Pinus* needles · Recovery of photosynthesis

Abbreviations AL: Actinic light · Chl: Chlorophyll · EPS: Epoxidation state of the xanthophyll cycle pigments · F_o: Instantaneous (dark) chlorophyll fluorescence at open PSII centers in dark-adapted samples · $F_{o'}$: Minimal fluorescence at open PSII centers immediately after illumination · F_m: Maximal fluorescence at closed PSII centers · $F_{m'}$: Maximal fluorescence at closed PSII centers under actinic light · F_v: Variable fluorescence · $F_{v'}$: Variable fluorescence under actinic light · $F_v/F_m = (F_m - F_o)/F_m$: The maximal photochemical efficiency of PSII · $F_{v'}/F_{m'} = (F_{m'} - F_{o'})/F_{m'}$: The efficiency of open PSII units under illumination · LHCII: The major Chl *a*/*b* pigment–protein complex associated with photosystem II · qN, NPQ: Nonphotochemical quenching · P700: Reaction center pigment of PSI · $P700^+$: Oxidized form of the reaction center of PSI · PSI, PSII: Photosystem I and photosystem II, respectively · PQ: Plastoquinone · Q_A: Primary electron-accepting quinone in PSII · Q_B: Secondary electron-accepting quinone in PSII · qP: Photochemical quenching · SN: Summer needles · WN: Winter needles

A. G. Ivanov · M. Krol · N. P. A. Huner (✉)
Department of Biology and the Biotron, University of Western Ontario, 1151 Richmond Str. N, London, ON, Canada N6A 5B7
E-mail: nhuner@uwo.ca
Tel.: +1-519-6792111
Fax: +1-519-6613935

A. G. Ivanov · D. Sveshnikov · G. Malmberg · P. Gardeström
V. Hurry · G. Öquist
Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 90187 Umea, Sweden

Introduction

Following the initial report of photosynthesis by bark chlorenchymes (Pearson and Lawrence 1958), photosynthetic activity in chlorophyll-containing stem and bark tissues has been reported in a wide variety of woody species (Adams and Strain 1969; Kriedemann and Buttrose 1971; Foote and Schaedle 1976; Pilarski 1995; Pfanz 1999; Schmidt et al. 2000; Wittmann et al.