Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 807–813
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbabio

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*

Received 27 October 2006; received in revised form 5 February 2007; accepted 6 February 2007
Available online 13 February 2007

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).

0005-2728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2007.02.006

induces a differential decrease in the PSI-associated fluorescence peak, and related PSI reaction center proteins [12] with a concomitant reduction in the activity of PSI [9,12] and replacement of ferredoxin with flavodoxin [12]. One of the most specific responses is the appearance of a Chl–protein complex CP43′ associated with the *isiA* gene product [16–18]. It has been demonstrated that the induction of CP43′ under Fe-stress causes the formation of a distinctive antenna ring of 18 molecules of CP43′ around trimeric PSI in cyanobacteria [19,20]. More recently, detailed structural analysis of PSI revealed the existence of various types of trimer and monomer PSI–IsiA supercomplexes [15], suggesting more dynamic and versatile role for the IsiA protein in light harvesting [19] and/or photoprotection [21,11] in response to Fe-stress. Our previous data also indicated that CP43′ is preferentially associated with PSI but this association is not restricted to PSI trimers since CP43′ is also detectable in PSI monomers [10]. In addition, we have shown that apart from the induction of CP43′, another major consequence of iron stress in cyanobacterial cells *in vivo* appears to be monomerization of PSI trimers and reduced capacity for state transitions [10].

Despite the extensive studies and important findings in recent years regarding the induction and regulation of *isiA* gene during iron stress and the structural and functional role of CP43′ protein in adaptation of cyanobacterial cells to iron deficient growth conditions, there are only a few studies addressing the response of carotenoid composition to iron stress [22,23]. More strikingly, apart from a recent study demonstrating decreased amount of PG in iron stresses *Synechococcus* cells [10], the effects of iron stress on lipid fatty acids composition in cyanobacteria have not been studied in detail, although the critical importance of membrane lipids for growth, respiration and photosynthesis of cyanobacteria [24] as well as their role in adaptation to salt stress [25], cold stress [26] and high light stress [27] have been well established. In this study we examine iron-stress induced alterations in the carotenoid and lipid composition of *Synechococcus* sp. PCC 7942 cells as well as their functional roles in photoprotection.

2. Materials and methods

2.1. Growth conditions

Synechococcus sp. PCC 7942 cells were grown axenically in liquid BG-11 inorganic medium, supplemented with 10 mM MOPS (pH 7.5). Cultures were grown in 80 ml of fresh growth medium in rod-shaped glass tubes, bubbled with 5% CO_2 in air at 35 °C with continuous illumination of 50 μmol photons m^{-2} s^{-1} of white light (400–700 nm) (Philips TLD 18W/950 fluorescent tubes) as in [9]. Iron limitation was achieved by culturing cells in a BG-11 medium lacking ferric ammonium citrate. The Chl concentration at the onset of all iron deficiency experiments was 2 μg ml^{-1} after inoculation in iron-deficient media. All experiments with iron stressed cells were performed after 72 h growth in iron-deficient media.

2.2. Lipid analysis

For lipid analysis, control and iron-deficient *Synechococcus* cells were collected by centrifugation at 4000×g_{max} for 15 min and frozen in liquid N_2 for later lipid extraction. The lipids were extracted according to Bligh and Dyer [28]. The individual lipids were isolated by two-dimensional TLC and quantified from their acyl group composition as previously described [29], except that the trans-esterification of the fatty acids were performed at 80 °C in 5% H_2SO_4 in dry methanol for 2 h.

2.3. Pigment analysis

Control and iron deficient *Synechococcus* cells were harvested by centrifugation and pigments were extracted with 100% acetone at 4 °C as in [30]. Pigments were separated and quantified by high-performance liquid chromatography (HPLC) using a system containing a Beckman System Gold programmable solvent module 126, diode array detector module 168 (Beckman Instruments, San Ramon, CA, USA), CSC-Spherisorb ODS-1 reverse phase column (5 μm particle size, 25×0.46 cm I.D.) with an Upchurch Perisorb guard column (both columns from Chromatographic Specialties Inc., Concord, ON, Canada) as described previously [30].

2.4. Non-denaturating SDS-PAGE

Cyanobacterial thylakoid membranes were prepared as in [31]. Electrophoretic separation of Chl–protein complexes was performed on an 8% (w/v) polyacrylamide resolving gel containing 150 mM Tris–HCl (pH 8.8) buffer, a 4% (w/v) stacking gel containing 50 mM Tris–HCl (pH 6.8) buffer and a running buffer contained 0.2% (v/v) Deriphat 160 [32] essentially as described in [10]. Samples were loaded with an equal amount of Chl. The excised bands were scanned at 671 nm on a Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA) for Chl absorbance and the relative content of each band was determined by the peak area normalized to the total area of the scan.

2.5. Chlorophyll fluorescence

Low temperature (77 K) fluorescence emission spectra of dark adapted intact cells were measured using a fiberoptic-based liquid nitrogen device attached to a Jobin Yvon FluoroMax-2 spectrofluorometer (ISA Jobin Yvon-Spex Instruments S.A., Longjumean, France) as described previously [12]. Cells with Chl concentration of 5 μg ml^{-1} were dark adapted at 35 °C for 30 min and frozen in the presence of 30% glycerol before the measurements. Chl *a* fluorescence was excited at 436 nm. Corrected fluorescence spectra were recorded from 670 nm to 760 nm. Exciting and measuring slits were 4 nm. All fluorescence spectra were corrected by subtracting the medium blank and were normalized to the PSII peak at 695 nm. Decomposition analysis of the spectra in terms of Gaussian bands was carried out by a non-linear least squares algorithm that minimises the chi-square function using a Microcal Origin Version 6.0 software package (Microcal Software Inc., Northampton, MA, USA). The fitting parameters for the four Gaussian components, that is, position, area and full width at the half maximum (FWHM), were free-running parameters.

2.6. Thermoluminescence

Thermoluminescence (TL) measurements of control and iron deficient wild type *Synechococcus* sp. PCC 7942 cells were performed on a personal-computer-based TL data acquisition and analysis system as described earlier [33]. The heating rate was 0.6 °C s^{-1}. The samples were cooled and illuminated with two successive single turnover flashes. For $S_2Q_A^-$ recombination studies, cells were incubated in the presence of DCMU in darkness before the illumination. When present, DCMU was at a final concentration of 10 μM in a cell suspension containing 0.2–0.3 mg Chl. The nomenclature of Vass and Govindjee [34] was used for characterisation of the TL glow peaks.

3. Results and discussion

Decomposition analysis of the 77 K fluorescence emission spectra of iron sufficient and iron stressed *Synechococcus* sp. PCC 7942 cells excited at 436 nm yielded a best fit with four spectral components (Fig. 1, Table 1) corresponding to the

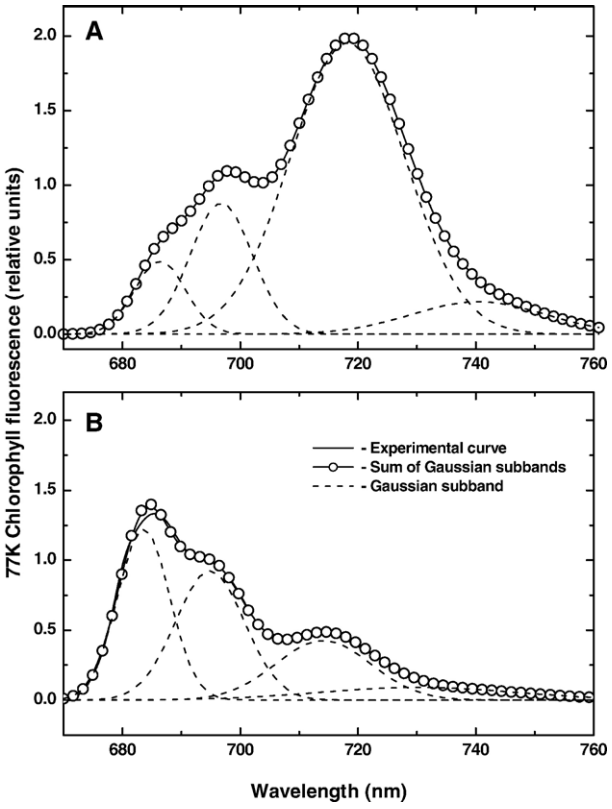

Fig. 1. Low temperature (77 K) fluorescence emission spectra and decomposition in Gaussian sub-bands of iron sufficient (A) and iron deficient (B) *Synechococcus* cells. Fluorescence was excited at 436 nm. Experimental curves represent averages of 3 scans. Spectra were normalized at the PSII peak at 695 nm. Experimental curve (solid line), sum of Gaussian sub-bands (open circles), Gaussian sub-bands (dashed lines).

emission of two sub-bands from PSII (683–686 and 694–696 nm), PSI (714–718 nm) [35] and a small spectral component (F_{vib}) centered around 730–740 nm enfolding all numbers of small vibrational transitions in the near-infrared region [36]. In agreement with earlier studies [18,21,12] low temperature (77 K) fluorescence measurements revealed typical response of *Synechococcus* cells to growth in iron depleted medium with the increased relative amplitude of the PSII associated peak centered at 685 nm and a decrease of the PSI associated peak at 718 nm (Fig. 1). Although the overall composition of spectral sub-bands in both iron sufficient and deficient cells was similar, the area ratios of the sum of PSII-related and PSI-related components is significantly higher in Fe-deficient cells (3.14) as compared to the control, Fe-sufficient cells (0.35) (Table 1). This indicates an imbalanced light energy distribution between the two photosystems in cells under low iron and is in agreement with previous data showing doubling in the ratio of PSII/PSI in iron-stressed versus control cultures of *Synechococcus* [9]. These data were accompanied by the appearance of the *isiA* gene product, the CP43′ chlorophyll-binding polypeptide typical for iron-stressed cells (data not shown), thus confirming the iron-stress response of *Synechococcus* cells under our experimental conditions.

In addition to a drastically reduced abundance of PSI reaction center proteins and the induction of CP43′ under Fe-stress [9,12], recent structural studies have demonstrated the formation of a variety of trimer and monomer PSI–CP43′ supercomplexes [19,20,15] which are associated with monomerization of PSI trimers [10]. The critical role of the PsaL subunit of PSI [37] and phosphatidylglycerol (PG) in the PsaL-dependent formation and stabilization of PSI trimers was recently established [38,39]. Indeed, our previous study demonstrated that monomerization of PSI trimers under iron stress is associated with a 50% decrease in the amount of PG concomitant with a 1.7- and 1.4-fold increase of DGDG and SQDG, respectively [10]. However, no detailed lipid analysis of the fatty acids composition has been ever presented in iron stressed cyanobacteria.

In the present study, the lipid analysis of control (Fe-sufficient) *Synechococcus* sp. PCC 7942 exhibited a similar distribution and composition of the lipid classes (data not shown) to that published earlier [40,41,29,10], and identical response to iron stress [10]. The fatty acid composition of the major lipid classes in control *Synechococcus* cells exhibited the same pattern with C16:0/C16:1 being the dominating fatty acid (around 85%, Tables 2 and 3) as previously reported [40,29]. In iron stressed cells the total amount of C18:0 and C18:1 almost doubled in all lipids except SQDG, while the amount of C16:0 and C16:1 fatty acids decreased indicating that the acylation at sn-1 position has changed. This was accompanied by significant desaturation of C18:0 to C18:1, thus increasing the amount of C18:1 in MGDG, DGDG and PG by 2.1-, 1.7- and 1.7-folds respectively (Table 2). As a result, the total unsaturation level was increased by about 5% in the major lipids (MGDG and DGDG) and 3% in PG (Table 3).

The higher fatty acid unsaturation level under iron stress conditions seems somewhat unexpected since desaturation of fatty acids requires ferredoxin (Fd) as electron donor for

Table 1
Gaussian parameters for the sub-band decomposition of low temperature (77 K) fluorescence emission spectra of *Synechococcus* cells grown under iron sufficient (+Fe) and iron deficient (−Fe) conditions

Parameters	*Synechococcus* sp. PCC 7942	
	+Fe	−Fe
1 λ max	686.39	683.60
FWHM	8.59	8.77
Area %	7.48	34.21
2 λ max	696.7	694.87
FWHM	10.55	11.70
Area %	16.45	34.34
3 λ max	718.24	714.08
FWHM	19.44	16.14
Area %	67.29	21.81
4 λ max	740.5	730.24
FWHM	22.8	33.31
Area %	8.76	9.61

The percentage areas of the spectral forms have been calculated from the total area given by the sum of all bands. The FWHM of each band is the sum of the left and right HWHM values. FWHM—full width at half maximum; HWHM—half width at half maximum.

Table 2
Fatty acid composition of the major membrane lipids of *Synechococcus* sp. PCC 7942 grown under control (+Fe) and iron deficient (−Fe) conditions, respectively

Sample	Fatty acid (mol%)					
	14:0	14:1	16:0	16:1	18:0	18:1
MGDG (+Fe)	1.4±0.3	1.3±0.2	48.2±0.6	37.8±1.0	3.3±0.5	7.7±0.7
MGDG (−Fe)	1.1±0.2	0.5±0.1	41.9±2.7	34.7±1.4	5.3±1.1	16.4±3.2
DGDG (+Fe)	2.1±0.4	0.4±0.2	50.5±1.6	33.9±0.7	3.3±0.5	9.4±0.7
DGDG (−Fe)	1.5±0.4	ND	44.7±1.6	33.2±0.9	4.8±0.5	15.6±2.2
SQDG (+Fe)	1.1±0.3	ND	57.6±2.3	31.8±0.7	1.5±0.3	7.5±2.2
SQDG (−Fe)	0.9±0.3	Tr	57.8±1.7	31.8±0.4	0.9±0.1	8.3±1.6
PG (+Fe)	0.6±0.1	ND	51.7±0.6	33.7±1.0	1.6±0.2	11.9±0.6
PG (−Fe)	0.8±0.3	ND	47.8±2.2	28.7±1.4	2.6±0.3	20.0±3.3

ND, not detected.
Tr, traces (<1.0%).
All values represent means±SE. (n=6).

desaturases, Fd-NADP$^+$ oxidoreductase and NADPH [42,43]. The iron containing Fd is replaced by flavodoxin (Fld) in iron stressed cyanobacteria [44,12]. However, it has been demonstrated that the enzyme Fd-NADP$^+$ oxidoreductase (FNR) forms a 1:1 complex with both electron transfer proteins, Fd and Fld and both proteins occupy the same region for the interaction with the reductase [45]. This suggests more effective electron transfer capacity of Fld under iron deficient conditions, which might facilitate the increased desaturation of membrane lipids under iron stress.

Since *Synechococcus* sp. PCC 7942 strain has only one known desaturase and the introduction of the first double bond into the fatty acid chain has the major impact on the phase transition temperature [46], the increased unsaturation implies increased membrane fluidity, which may facilitate the diffusion of PQ and keep the PQ pool in relatively more oxidized state in iron-stressed compared to control cells. Indeed, chlorophyll fluorescence measurements revealed higher capacity of iron stressed cells to keep the PQ pool in a relatively more oxidized state despite the fact that the abundance of PSI is drastically reduced under the same conditions [10].

Table 3
Fatty acids (FA) chain length distribution and FA unsaturation (Total C:1 FA) in control (+Fe) and iron deficient (−Fe) *Synechococcus* sp. PCC 7942 cells

Lipids	C_{14} FA	C_{16} FA	C_{18} FA	Unsaturation (Total C:1 FA)
MGDG (+Fe)	2.7	86.0	11.0	46.8
MGDG (−Fe)	1.6	76.6	21.7	51.6
DGDG (+Fe)	2.5	84.4	12.7	43.7
DGDG (−Fe)	1.5	77.9	20.4	48.8
SQDG (+Fe)	1.1	89.4	9.0	39.3
SQDG (−Fe)	0.9	89.6	9.2	40.1
PG (+Fe)	0.6	85.4	13.5	45.6
PG (−Fe)	0.8	76.5	22.6	48.7

Means values represent averages from 6 independent experiments.

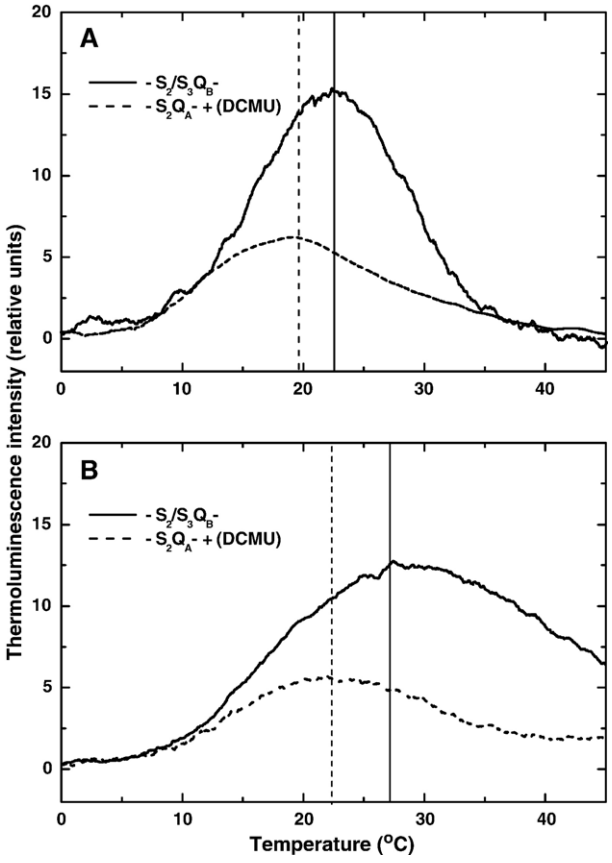

Fig. 2. Thermoluminescence glow curves of *Synechococcus* sp. PCC 7942 cells under control (A) and iron deficient (B) growth conditions. Solid lines—glow curves of $S_2/S_3Q_B^-$ charge recombinations, dashed lines—glow curves of $S_2Q_A^-$ charge recombinations in the presence of DCMU (10 μM). The presented glow curves are averages from 3 to 5 measurements in 3 independent experiments.

Additional support for the altered redox state of the electron transport components in iron deficient *Synechococcus* cells was provided by TL measurements (Fig. 2). Concomitant with previous results [33] the TL glow curve of control (iron sufficient) cells exhibited a peak with characteristic T_M of 22.5 °C (Fig. 2A) corresponding to $S_2/S_3Q_B^-$ charge recombination [33,47]. In the presence of DCMU the overall thermoluminescence emission was much lower and the new peak exhibiting T_M of 18.5°C was assigned to $S_2Q_A^-$ charge recombination respectively [33]. In iron deficient cells the characteristic temperatures of both $S_2/S_3Q_B^-$ and $S_2Q_A^-$ peaks were shifted to higher temperatures (29.0 °C and 22.2 °C), respectively (Fig. 2B).

Apart from other mechanisms, the downshift of the $S_2Q_B^-$ T_M has also been attributed to the build-up of a dark-induced proton gradient and the reduction of the PQ pool [48]. It has been demonstrated that the reduction of PQ pool affects both Q ($S_2Q_A^-$) and B ($S_2Q_B^-$) TL bands in a similar manner [48]. Hence, it seems reasonable to suggest that the shift of T_M assigned to $S_2Q_A^-$ and $S_2Q_B^-$ to higher temperatures is due to the fact that in iron deficient cells PQ pool is relatively more oxidized.

The photosynthetic and respiratory electron transfer chains of cyanobacteria share common redox components [49] and it is generally accepted that the redox state of the PQ pool is

Table 4
Effect of iron deficiency on the carotenoids composition of *Synechococcus* sp. PCC 7942 cells

Carotenoids (μM/μM Chl *a*)	*Synechococcus* sp. PCC 7942	
	+Fe	−Fe
Nostoxanthin	0.013±0.003	0.034±0.004
Cryptoxanthin	0.028±0.004	0.089±0.009
Caloxanthin	0.289±0.016	0.383±0.022
Zeaxanthin	0.042±0.004	0.080±0.006
β-carotene	0.210±0.0119	0.400±0.018

Mean values±SE were calculated from 3 independent experiments.

controlled not only by the photosynthetic electron transport, but also by the respiratory electron flow into the PQ pool mediated by the NAD(P)H-dehydrogenase complex. Thus, in addition to facilitated PQ diffusion due to higher unsaturation of membrane lipids, the lower abundance of the NAD(P)H-dehydrogenase complex in cells grown under Fe-stress conditions [10] may also contribute to the observed higher oxidized state of PQ in iron stressed *Synechococcus* cells.

The energy dissipating role of IsiA protein in photoprotection of cyanoobacteria under iron stress has been suggested [21,11,15]. However, although the photoprotective role assigned to protein–pigment complexes could not be achieved without the presence of carotenoids, qualitative and quantitative data on carotenoid composition of cyanobacteria under iron deficient conditions are limited and contradictory. While decreased availability of Fe(III) in *Synechococcus* sp. PCC 7002 caused significant reduction of β-carotene and zeaxanthin content [22], cultivation of *Synechococcus elongatus* in iron-deficient media resulted in much higher relative concentrations of all carotenoids [23].

Analysis of the HPLC-separated pigments of control (iron sufficient) cells exhibited a carotenoid composition typical for *Synechococcus* sp. PCC 7942 cells consisting of nostoxanthin, cryptoxanthin, zeaxanthin, caloxanthin and β-carotene. The quantities of carotenoids calculated on a Chl basis (Table 4) were well within the range of previously reported data for *Synechococcus* strains [50,23]. A comparison of carotenoid composition of control to that of iron-stressed cells indicated an accumulation of all carotenoids relative to Chl *a*. The relative amounts of carotenoids 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% (Table 4).

Non-denaturating PAGE of thylakoid membranes from control (Fig. 3A) and Fe-stressed (Fig. 3B) *Synechococcus*

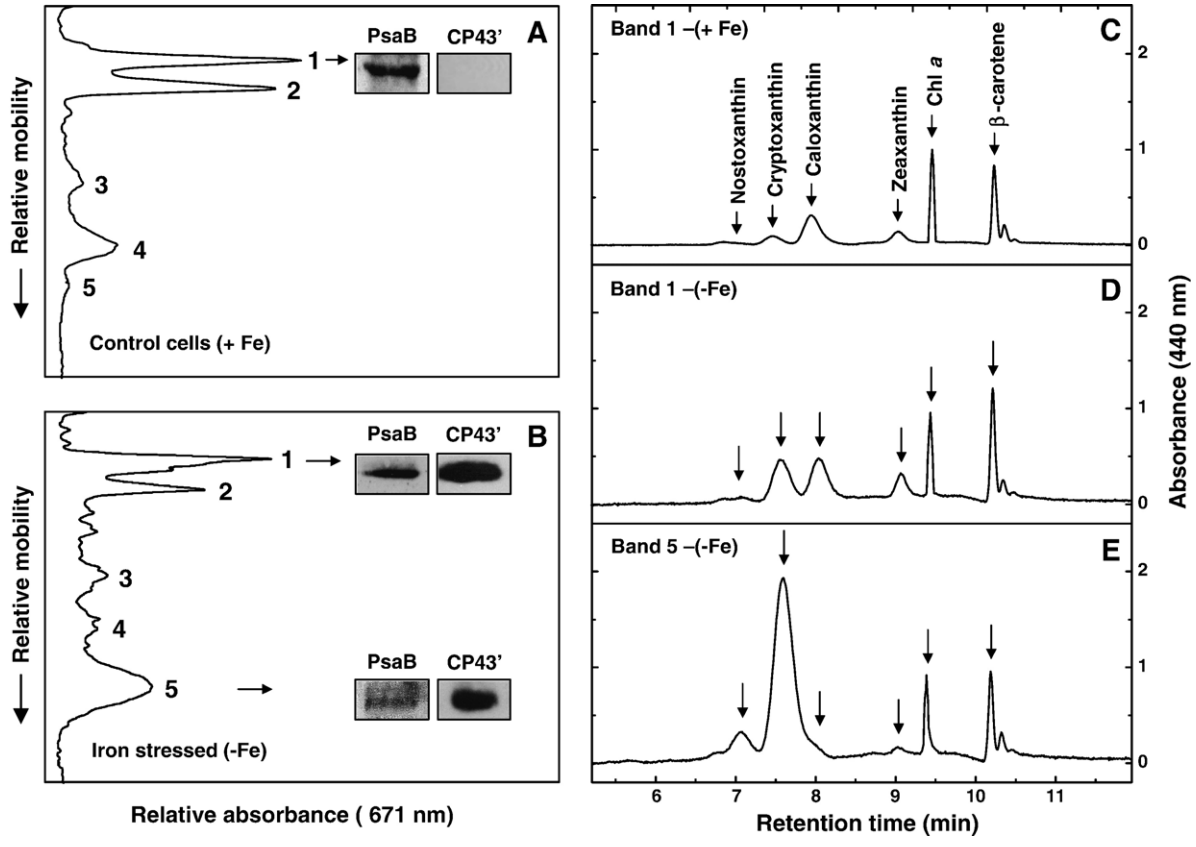

Fig. 3. Densitograms of Chl–protein complexes (1–5) separated by non-denaturating PAGE in thylakoid membranes of control (A) and Fe- stressed (B) *Synechococcus* cells. Band 1 is designated to the PSI trimer (PsaA/PsaB) core complex; band 2—PSII (D1+CP43) reaction center complex, band 3—a mixture of PSII and PSI, band 4—PSII (D1+CP43) complex, band 5—PsaA/PsaB+CP43′. The traces in A and B represent averages from 3 to 5 independent experiments. C–E—typical HPLC chromatograms representing the pigment composition of band 1 in control cells and band 1 and 5 in Fe-stressed cells, respectively. All chromatograms are normalized to the corresponding Chl *a* peaks. The insets in A and B represent immunoblots of PsaB and CP43′ polypeptides of Chl–protein complexes in band 1 and band 5 separated by non-denaturating PAGE in control and Fe-deficient *Synechococcus* cells, respectively.

cells resolved six distinct bands associated with the major Chl–protein complexes of PSI and PSII: PSI trimer (PsaA/PsaB) core complex and trimer PSI–CP43′ supercomplex (band 1), PSII (D1+CP43) reaction center complex (band 2), a mixture of PSII and PSI (band 3), PSII (D1+CP43) complex (band 4), PSI monomer (PsaA/PsaB)+CP43′ (band 5) and free pigments (band 6, not shown) [32,51,10]. The most distinctive differences between the control and Fe-stressed cells was the appearance of a new peak (band 5) and decreased relative abundance of the PSI trimer (band 1) in iron-stressed cells (Fig. 3B). As expected, PsaB was detected by immunoblotting in band 1 (PSI trimers) of control and in bands 1 (PSI trimers) and 5 (PSI monomers) in iron stressed cells (Fig. 3, insets), while CP43′ protein was detected only in bands 1 and 5 of thylakoid membranes isolated from iron-stressed cells (Fig. 3B, insets) [10]. It is important to note that the CP43′ protein in Fe-stressed cells was found to co-migrate with PsaB and was detected in both band 1 and band 5 designated to PSI-trimer and PSI-monomer, respectively [10].

The individual bands of the PSI-related Chl–protein complexes were excised from the gels and pigments were extracted and separated by HPLC chromatography. Typical HPLC chromatograms of PSI complexes from control (iron sufficient) and iron stressed cells are presented in Fig. 3C–E. The chromatograms are normalized to the Chl *a* peak in each band. The qualitative carotenoid composition of PSI trimer complex in control cells (Fig. 3C) fully resembles that of control cells (Table 4). In the PSI–CP43′ trimer complex from iron-stressed cells the relative amounts of all carotenoids, especially that of cryptoxanthin were increased (Fig. 3D) and exceeded that registered in whole cells (Table 4). More interestingly, PSI–CP43′ monomer band (band 5) exhibited even higher increase of cryptoxanthin and nostoxanthin, while caloxanthin, zeaxanthin and β-carotene peaks were lower (Fig. 3E) compared to that in PSI–CP43′ trimer band (Fig. 3D) of iron stressed cells. Unfortunately, quantitative carotenoid analysis of PSI and PSI–CP43′ complexes is not available from our study, because the precise polypeptide composition of the bands corresponding to these complexes separated by non-denaturating SDS-PAGE is not known and may vary. However, considering that the major differences between the PSI complexes is the association of both PSI-trimers and PSI-monomers in iron stressed cells with CP43′ as compared to control cells a carotenoid-binding role for CP43′ protein could be suggested. Indeed, recent study has indicated that IsiA (CP43′) protein from iron-stressed *Synechocystis* sp. PCC 6803 binds zeaxanthin and echinenone [52]. This strengthens earlier reports indicative of effective energy quenching and photoprotective role of CP43′ protein in cyanobacteria under iron stress [21,11].

Acknowledgments

This work was financially supported by grants from the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) to GÖ and NPAH, the Swedish Research Council to GÖ and the Natural Science and Engineering Research Council of Canada to NPAH.

References

[1] J.H. Martin, M.R. Gordon, S.E. Fitzwater, The case for iron, Limnol. Oceanogr. 36 (1991) 1793–1802.
[2] J.A. Raven, The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources, New Phytol. 109 (1988) 279–287.
[3] J.A. Raven, Predictions of Mn and Fe use efficiencies of photoautotrophic growth as a function of light availability for growth and C assimilation pathway, New Phytol. 116 (1990) 1–18.
[4] F. Ferreira, N.A. Straus, Iron deprivation in cyanobacteria, J. Appl. Phycol. 6 (1994) 199–210.
[5] M.J. Behrenfeld, A.J. Bale, Z.S. Kolber, J. Aiken, P.G. Falkowski, Confirmation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean, Nature 283 (1996) 508–511.
[6] D.M. Sherman, L.A. Sherman, Effect of iron deficiency and iron restoration on ultrastructure of *Anacystis nidulans*, J. Bacteriol. 156 (1983) 393–401.
[7] G. Öquist, Iron deficiency in the blue-green alga *Anacystis nidulans*: fluorescence and absorption spectra recorded at 77K, Physiol. Plant. 31 (1974) 55–58.
[8] J.A. Guikema, L.A. Sherman, Organization and function of chlorophyll in membranes of cyanobacteria during iron starvation, Plant Physiol. 73 (1983) 250–256.
[9] A.G. Ivanov, Y.-I. Park, E. Miskiewicz, J.A. Raven, N.P.A. Huner, G. Öquist, Iron stress restricts photosynthetic intersystem electron transport in *Synechococcus* sp. PCC 7942, FEBS Lett. 485 (2000) 173–177.
[10] A.G. Ivanov, M. Krol, D. Sveshnikov, E. Selstam, S. Sandström, M. Koochek, Y.-I. Park, S. Vasil'ev, D. Bruce, G. Öquist, N.P.A. Huner, Iron deficiency in cyanobacteria causes monomerization of PSI trimers and reduces the capacity for state transitions and the effective absorption cross section of PSI *in vivo*, Plant Physiol. 141 (2006) 1436–1445.
[11] S. Sandström, Y.-I. Park, G. Öquist, P. Gustafsson, CP43′, the *isiA* gene product, functions as an energy dissipator in the cyanobacterium *Synechococcus* sp. PCC 7942, Photochem. Photobiol. 74 (2001) 431–437.
[12] S. Sandström, A.G. Ivanov, Y.-I. Park, G. Öquist, P. Gustafsson, Iron stress responses in the cyanobacterium *Synechococcus* sp. PCC 7942, Physiol. Plant. 116 (2002) 255–263.
[13] N.A. Straus, Iron deprivation: physiology and gene regulation, in: D.A. Bryant (Ed.), The Molecular Biology of Cyanobacteria, Kluwer Academic Publishers, Dordrecht, 1994, pp. 731–750.
[14] K.-P. Michel, E.K. Pistorius, Adaptation of the photosynthetic electron transport chain in cyanobacteria to iron deficiency: the function of IdiA and IsiA, Physiol. Plant. 120 (2004) 36–50.
[15] R. Kouřil, A.A. Arteni, J. Lax, N. Yeremenko, S. D'Haene, M. Rögner, H.C.P. Mattijs, J.P. Dekker, E.J. Boekema, Structure and functional role of supercomplexes of IsiA and photosystem I in cyanobacterial photosynthesis, FEBS Lett. 579 (2005) 3253–3257.
[16] H.B. Pakrasi, H.C. Reitham, L.A. Sherman, Organization of pigment proteins in the photosystem II complex of the cyanobacterium *Anacystis nidulans* R2, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 6903–6907.
[17] H.C. Riethman, L.A. Sherman, Purification and characterization of an iron stress-induced chlorophyll–protein from the cyanobacterium *Anacystis nidulans* R2, Biochim. Biophys. Acta 935 (1988) 141–151.
[18] R.L. Burnap, T. Troyan, L.A. Sherman, The highly abundant chlorophyll–protein of iron deficient *Synechococcus* sp. PCC 7942 (CP43′) is encoded by the isiA gene, Plant Physiol. 103 (1993) 893–902.
[19] E.J. Boekema, A. Hifney, A.E. Yakushevska, M. Piotrowski, W. Keegstra, S. Berry, K.-P. Michel, E.K. Pistorius, J. Kruip, A giant chlorophyll–protein complex induced by iron deficiency in cyanobacteria, Nature 412 (2001) 745–748.
[20] T.S. Bibby, J. Nield, J. Barber, Iron deficiency induces the formation of an antenna ring around trimeric photosystem I in cyanobacteria, Nature 412 (2001) 743–745.
[21] Y.-I. Park, S. Sandström, P. Gustafsson, G. Öquist, Expression of the *isiA* gene is essential for the survival of the cyanobacterium *Synechococcus* sp. PCC 7942 by protecting photosystem II from excess light under iron limitation, Mol. Microbiol. 32 (1999) 123–129.

[22] S.W. Wilhelm, D.P. Maxwell, C.G. Trick, Growth, iron requirements, and siderophore production in iron-limited *Synechococcus* PCC 7002, Limnol. Oceanogr. 41 (1996) 89–97.
[23] J. Benešová, K. Ničková, N. Ferimazova, D. Štys, Morphological and physiological differences in *Synechococcus elongatus* during continuous cultivation at high iron, low iron, and iron deficient medium, Photosynthetica 38 (2000) 233–241.
[24] Y. Tasaka, Z. Gombos, Y. Nishiyama, P. Mohanty, T. Ohba, K. Ohki, N. Murata, Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis*: evidence for the important roles of polyunsaturated membrane lipids in growth, respiration and photosynthesis, The EMBO J. 15 (1996) 6416–6425.
[25] S.I. Allakhverdiev, Y. Nishiyama, I. Suzuki, Y. Tasaka, N. Murata, Genetic engineering of the unsaturation of fatty acids in membrane lipids alters the tolerance of *Synechocystis* to salt stress, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 5862–5867.
[26] H. Wada, Z. Gombos, N. Murata, Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation, Nature 347 (1990) 200–203.
[27] Z. Gombos, E. Kanervo, N. Tsvetkova, T. Sakamoto, E.-M. Aro, N. Murata, Genetic enhancement of the ability to tolerate photoinhibition by introduction of unsaturated bonds into membrane glycerolipids, Plant Physiol. 115 (1997) 551–559.
[28] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[29] J. Porankiewicz, E. Selstam, D. Campbell, G. Öquist, Membrane lipid composition and restoration of photosynthesis during low temperature acclimation of *Synechococcus* sp. strain PCC 7942, Physiol. Plant 104 (1998) 405–412.
[30] A.G. Ivanov, E. Miskiewicz, A.K. Clarke, B.M. Greenberg, N.P.A. Huner, Protection of photosystem II against UV-A and UV-B radiation in the cyanobacterium *Plectonema boryanum*: the role of growth temperature and growth irradiance, Photochem. Photobiol. 72 (2000) 772–779.
[31] G.M. Giacometti, R. Barbato, S. Chiaramonte, G. Friso, F. Rigoni, Effects of ultraviolet-B radiation on photosystem II of the cyanobacterium *Synechocystis* sp. PCC 6083, Eur. J. Biochem. 242 (1996) 799–806.
[32] J. Komenda, Role of two forms of the D1 protein in the recovery from photoinhibition of photosystem II in the cyanobacterium *Synechococcus* PCC 7942, Biochim. Biophys. Acta 1457 (2000) 243–252.
[33] P.V. Sane, A.G. Ivanov, D. Sveshnikov, N.P.A. Huner, G. Öquist, 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 QB, J. Biol. Chem. 277 (2002) 32739–32745.
[34] I. Vass, Govindjee, Thermoluminescence from the photosynthetic apparatus, Photosynth. Res. 48 (1996) 117–126.
[35] D.C. Fork, P. Mohanty, Fluorescence and other characteristics of blue green algae (cyanobacteria), red algae and cryptomonads, in: Govindjee, J. Ametz, D.C. Fork (Eds.), Light Emission by Plants and Bacteria, Academic Press, Orlando, 1986, pp. 451–496.
[36] R. Van Grondelle, J.P. Dekker, T. Gillbro, V. Sundstrom, Energy-transfer and trapping in photosynthesis, Biochim. Biophys. Acta 1187 (1994) 1–65.
[37] V.P. Chitnis, P.R. Chitnis, PsaL subunit is required for the formation of photosystem I trimers in the cyanobacterium *Synechocystis* sp. PCC 6803, FEBS Lett. 336 (1993) 330–334.
[38] N. Sato, K. Suda, M. Tsuzuki, Responsibility of phosphatidylglycerol for biogenesis of the PSI complex, Biochim. Biophys. Acta 1658 (2004) 235–243.
[39] I. Domonkos, P. Malec, A. Sallai, L. Kovács, K. Itoh, G. Shen, B. Ughy, B. Bogos, I. Sakurai, M. Kis, K. Strzalka, H. Wada, S. Itoh, T. Farkas, Z. Gombos, Phosphatidylglycerol is essential for oligomerization of photosystem I reaction center, Plant Physiol. 134 (2004) 1471–1478.
[40] N. Murata, H. Wada, Z. Gombos, Modes of fatty-acid desaturation in cyanobacteria, Plant Cell Physiol. 33 (1992) 933–941.
[41] P. Deshnium, Z. Gombos, Y. Nishiyama, N. Murata, The action in vivo glycine betaine in enhancement of tolerance of *Synechocystis* sp. strain PCC 7942 to low temperature, J. Bacteriol. 179 (1997) 339–344.
[42] H. Schmidt, E. Heinz, Involvement of ferredoxin in desaturation of lipid-bound oleate in chloroplasts, Plant Physiol. 94 (1990) 214–220.
[43] H. Wada, H. Schmidt, E. Heinz, N. Murata, In vitro ferredoxin-dependent desaturation of fatty acids in cyanobacterial thylakoid membranes, J. Bacteriol. 175 (1993) 544–547.
[44] G.N. Hutber, K.G. Hutson, L.J. Rogers, Effect of iron deficiency on levels of two ferredoxins and flavodoxin in a cyanobacterium, FEMS Microbiol. Lett. 1 (1977) 193–196.
[45] M. Martinez-Julvez, M. Medina, C. Gomez-Moreno, Ferredoxin-NADP (+) reductase uses the same site for the interaction with ferredoxin and flavodoxin, J. Biol. Inorg. Chem. 4 (1999) 568–578.
[46] R.N.A.H. Lewis, D.A. Mannock, R.N. McElhaney, Membrane lipid molecular structure and polymorphism, in: R.M. Epand (Ed.), Current Topics in Membranes, vol. 44, Academic Press, San Diego, CA, 1997, pp. 25–102.
[47] L. Lupinkova, J.G. Metz, B.A. Diner, I. Vass, J. Komenda, Histidine residue 252 of the photosystem II polypeptide is involved in a light-induced cross-linking of the polypeptide with the α subunit of cytochrome *b*-559: study of a site-directed mutant of *Synechocystis* PCC 6803, Biochim. Biophys. Acta 1554 (2002) 192–201.
[48] T. Miranda, J.-M. Ducruet, Effects of dark- and light-induced proton gradients in thylakoids on the Q and B thermoluminescence bands, Photosynth. Res. 43 (1995) 251–262.
[49] S. Scherer, Do photosynthetic and respiratory electron transport chains share redox proteins? Trends Biochem. Sci. 15 (1990) 458–462.
[50] Z. Gombos, L. Vigh, Primary role of the cytoplasmic membrane in thermal acclimation evidenced in nitrate-starved cells of the blue-green alga, *Anacystis nidulans*, Plant Physiol. 80 (1986) 415–419.
[51] D.L. Tucker, L.A. Sherman, Analysis of chlorophyll–protein complexes from the cyanobacterium *Cyanothece* sp. ATCC 51142 by non-denaturing gel electrophoresis, Biochim. Biophys. Acta 1468 (2000) 150–160.
[52] J.A. Ihalainen, S. D'Haene, N. Yeremenko, H. van Roon, A.A. Arteni, E.J. Boekema, R. van Grondelle, H.C.P. Mattijs, J.P. Dekker, Aggregates of the chlorophyll-binding protein IsiA (CP43′) dissipate energy in cyanobacteria, Biochemistry 44 (2005) 10846–10853.