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Stromal electron flow through the plastoquinone pool controls the state transition capacity in *Synechococcus* sp. PCC 7942 cells. Effects of iron deficiency.

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Introduction

It has been generaly accepted that state transitions in cyanobacteria strongly depend and are triggered by changes in the redox state of an electron transport component closely associated with both the photosynthetic and respiratory electron transport chains (Mullineaux and Allen 1990). It has been also well established that dark-adapted cyanobacterial cells are usually in State II characterised by low fluorescence yield of PSII related components (Mullineaux and Allen 1990; Falk et al. 1995). In contrast, in response to limited iron supply, cells are locked in State I characterised by drastic increase of the low temperature (77K) fluorescence of PSII at 685 nm and sharp decrease of PSI related fluorescence at 715 nm (Oquist 1974; Falk et al. 1995). The specific 77K fluorescence pattern in iron starved cells is accompanied and closely associated with the appearance of a specific iron stress induced, PSII chlorophyll-protein complex CP43' (Pakrasi et al. 1985; Burnap et al. 1993).

Since the photosynthetic and respiratory electron transfer chains of cyanobacteria share common redox components (Scherer 1990), we address the question whether NAD(P)H dehydrogenase-mediated stromal electron pathway (Mi et al.1992) may play significant role in regulating the state transitions under normal and iron limited conditions

Materials and methods

Cell culture – Axenic cultures of control and iron stressed *Synechococcus* sp. PCC 7942 cells were grown in BG-11 inorganic medium as described previously (Ivanov et al., 2000a).

77K fluorescence - Low temperature (77K) chlorophyll fluorescence measurements were performed by using a fiberoptic-based liquid nitrogen device attached to a Jobin Yvon FluoroMax-2® spectrofluorimeter (ISA® Jobin Yvon-Spex Instruments S.A., Longjumean, France). Cells were dark adapted at 37°C for 30 min before the measurements. Corrected fluorescence spectra were recorded from 650 nm to 750 nm. Chlorophyll fluorescence was excited at 436 nm. Exciting and measuring slits were 4 nm.

Modulated Chl fluorescence - Chl *a* fluorescence was measured using a PAM 101 pulse amplitude modulated fluorescence measuring system described in (Ivanov et al.

2000b). Reduction state of PQ was assessed following the post-illumination transient increase of chlorophyll fluorescence at the F_0' level (Mano et al. 1995).

SDS-PAGE and immunoblotting. Protein samples containing equal amount of protein were separated on 15% linear polyacrylamide gels and electrophoretically transferred onto nitrocellulose membranes as described earlier (Morgan et al. 1998). Immunoblot analysis was performed as described in (Celerin et al. 1998). The CP43, CP43' and NDH-H proteins were detected with specific polyclonal antibodies at the following dilutions: CP43, 1:4000; CP43', 1:4000; NDH-H, 1:750.

Results and Discussion

Modulated chlorophyll fluorescence measurements have indicated that dark-adapted cyanobacterial cells are usually in State II characterised by low PSII fluorescence yield. Upon illumination, the quenching of PSII was released and fluorescence increased to a much higher level characteristic for cells in State I (Fig. 1A). In contrast, iron deficient cells exhibited higher PSII fluorescence in dark-adapted state and no fluorescence changes upon illumination were registered (Fig. 1C).

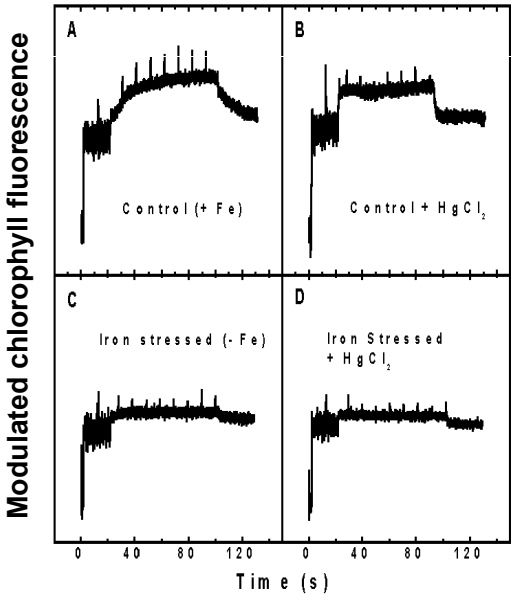

Fig. 1. Typical modulated chlorophyll fluorescence traces obtained from control (A, B); and iron stressed (C, D) *Synechococcus* sp. PCC 7942 cells. Non-treated cells – A, C; $HgCl_2$ (40 µmol)-treated cells – B, D. F_oL - measuring modulated light (650 nm, 0.12 µmol m^{-2} s^{-1}); SL - saturated light pulse (0.8 s, 2800 µmol $m^{-2}s^{-1}$); Actinic light (50 µmol $m^{-2}s^{-1}$). For all other experimental conditions see Materials and Methods.

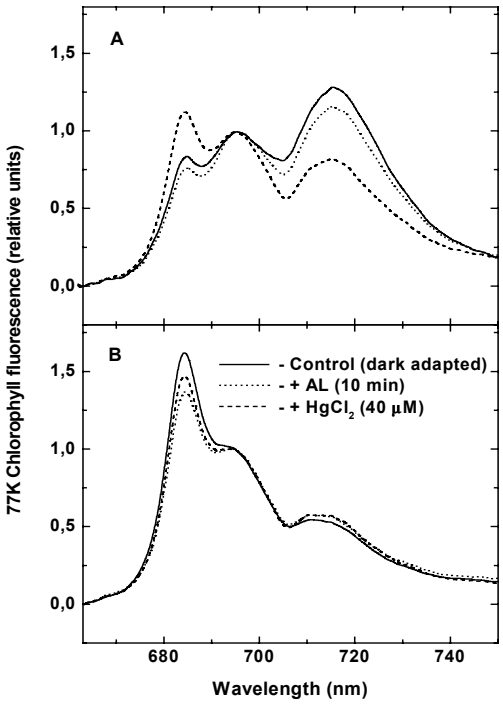

Fig. 2. Effect of $HgCl_2$ on the low temperature (77K) fluorescence emission spectra of control (A) and iron stressed (B) *Synechococcus* sp. PCC 7942 cells after a shift from control BG-11 medium to iron deficient conditions. All fluorescence spectra were normalised to the peak centred at 695nm.

As suggested earlier (Falk et al. 1995), this indicates that the cells are locked in State I under iron deficient conditions. Low temperature (77K) fluorescence measurements confirmed this suggestion. Emission peaks centred at 685nm and 695 nm assigned to

the PSII antenna and PSII core complexes were suppressed in control cells (Fig. 2A), while in iron stressed cells the PSII characteristic peaks were strongly increased and PSI peak was reduced (Fig. 2B).

Control iron sufficient cells exhibited a significant postillumination increase of Fo, which is a measure of the dark reduction of the PQ pool by the stromal electron flow (Fig. 3A) (Mano et al. 1995). Application of FR light during the postillumination period almost completely removed the transient increase of Fo in control cells (Fig. 3B). On the contrary, no measurable fast postillumination transient of Fo corresponding to dark reduction of the PQ pool was detected in iron deficient cells in the absence or presence of FR light (Fig. 3C, D).

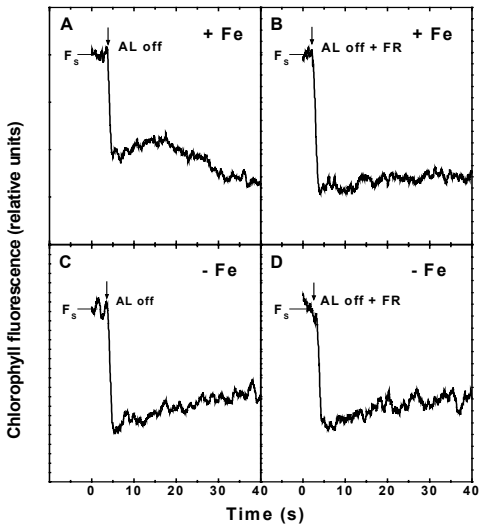

Fig. 3. The post-illumination transients from F_s to F_o' after the actinic light (AL, 50 µmol photons m^{-2} s^{-1}, 8 min) was turned off in *Synechococcus* sp. PCC 7942 cells grown under iron sufficient (+Fe) (A, B) and iron deficient (-Fe) (C, D) conditions. The intensity of far red (FR) light applied after turning off the AL was 10 W m^{-2} (B, D).

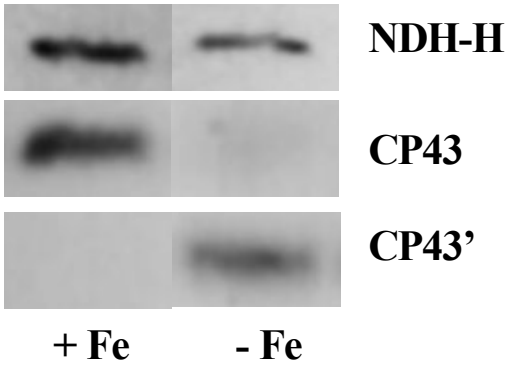

Fig. 4. Western blots of SDS-PAGE separated polypeptides from iron sufficient (+ Fe) and iron deficient (- Fe) *Synechococcus* cells probed with antibodies raised against CP43 (A), CP43' (B) and Cyt *f* (C). Numbers on the left represent molecular masses of markers.

Immunoblot analysis of SDS-PAGE of *Synechococcus* cells grown under iron deficiency revealed the appearance of a CP43' polypeptide typical for low iron acclimated cells (Pakrasi et al. 1985; Falk et al. 1995; Ivanov et al. 2000) associated with minor levels of CP43 polypeptide as compared to iron sufficient cells (Fig. 4). Concomitantly, iron deficient cells exhibited much lower levels of NAD(P)H dehydrogenase than control cells (Fig. 4).

Interestingly, preventing the stromal electron flow to the intersystem chain by using $HgCl_2$ as a specific inhibitor of NADP dehydrogenase (Ivanov et al. 2000), completely inhibited the light induced increase of PSII fluorescence in control cells (Fig. 1B). Fluorescence measurements at 77K indicated that under these dark-adapted conditions, control *Synechococcus* cells shifted from State II to State I and the

observed 77K fluorescence pattern resembled that of iron deficient cells (Fig. 2A). Based on these results, it is reasonable to suggest that the state transition phenomena in *Synechococcus* strongly depend on the redox state of the PQ pool and that the NADP-dependent stromal electron flow is one of the major mechanisms controlling it. Since the NADP-dehydrogenase is one of the most Fe-abundant complexes within the cyanobacterial thylakoid membranes which is strongly down regulated under iron limited conditions (Fig. 4), minimal stromal electron flow should be expected. This would prevent the PQ pool from dark reduction and keep the iron deficient cells in state I.

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