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CP43' induced under Fe-stress in *Synechococcus* sp. PCC 7942 is associated with PSI

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Introduction

Cells of *Synechococcus* sp. PCC 7942 grown under iron limited conditions show decreased phycocyanin and chlorophyll content, a characteristic blue shift of the main red maximum of Chl absorption, replacement of ferredoxin by flavodoxin, and the induction of a new chlorophyll-protein complex, CP43´ (Öquist, 1971; Öquist, 1974; Guikema and Sherman, 1983). According to its high homology to CP43, an established component of the PSII core, CP43' is usually considered to be a constituent of PSII. It has been suggested earlier that CP43' could possibly replace CP43 in iron stressed cells acting as an alternative antenna complex for PSII (Pakrasi et al., 1985) or as a reservoir for chlorophyll storage (Burnap et al., 1993). More recently, we have shown that CP43´ has a role as quencher with the ability to protect PSII from photoinhibitory damage during iron stress (Park et al., 1999). In addition, while CP43' is accumulated during iron stress, the decrease of initial CP43 is not so pronounced, which makes its functional substitution in the PSII core quite unlikely (Sandström et al., 2001b).

Since the precise localization and possible function of CP43' is still a matter of debate we address this question by examining the localization of CP43' within the chlorophyll-protein complexes of iron stressed *Synechococcus* cells separated by a non-denaturating green gel system and sucrose density gradient centrifugation.

Materials and methods

Cell culture growth - *Synechococcus* sp. PCC 7942 was grown in BG-11 inorganic medium as described previously (Ivanov et al., 2000). Cultures were grown for 5 days at A_{750}=0.3–0.4, and pelleted by 10 min centrifugation at 8,500 g and 4°C.
Preparation of thylakoids - Thylakoid preparations were performed as in (Kuhl et al., 2000). Cells were washed once in 100 ml of buffer (20 mM MES, pH 6.5, 10 mM $MgCl_2$, 10 mM $CaCl_2$), and resuspended in the same buffer with addition of 0.5 M mannitol and 1g/l lysozyme. After dark incubation at 39°C for 90 min on a shaker, cells were disrupted by a French press, and 100 µg/ml DNAse and 15 µg/ml RNAse were added to the extract. The thylakoids were pelleted by 10 min centrifugation at 15,000 g and 4°C, rinsed twice in washing buffer with mannitol, frozen in liquid N_2 with addition of 20% glycerol, and stored at –80°C.

Non-denaturating SDS-PAGE – Thawed thylakoid membranes were washed once with 50 mM Tricine (pH 8.0) at 4°C. The pellet was resuspended in a 0.3 M Tris-HCl (pH 7.5) solubilization buffer, containing 13% glycerol, 0.1% SDS, and 0.45% n-dodecyl β-D-maltoside, with a total detergent to chlorophyll ratio of 20:1. Non-denaturating electrophoresis was performed on a 12.5% (w/v) polyacrylamide resolving gel buffered with 375 mM Tris (pH 8.8) and a 4% (w/v) stacking gel buffered with 125 mM Tris (pH 6.8). The running buffer contained 0.2% Deriphat-160 (disodium-N-lauryl iminodipropionate). The excised lanes were scanned at 671 nm on a Beckman DU 640 spectrophotometer for Chl absorbance.

Sucrose density gradient - For separation over sucrose gradients, thylakoids were solubilized in 1% n-dodecyl β-D-maltoside (DDM:Chl ratio of 30:1) for 30 min at room temperature, and the supernatant was resolved over the 0-50% step sucrose gradients at 70,000 g for 12 hours. The gradients were based on 25 mM MES (pH 6.0), 10 mM NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 2 M glycine-betaine and 0.03% n-dodecyl β-D-maltoside as in (Eshaghi et al., 1999).

Protein analysis – Excised green gel bands and sucrose fractions were subjected to SDS-PAGE (Laemmli, 1970) and Western blotting, with ECL detection system (Amersham Pharmacia Biotech, UK).

Results

Non-denaturating SDS-PAGE of thylakoid membranes from *Synechococcus* cells gave five distinct bands (Fig. 1A). Densitometric scans of the gels exhibited considerable differences of the chlorophyll-protein complexes composition between the control and iron stressed cells (Fig. 1B). As expected, CP43 was detected by immunoblot analysis in the same bands where the PSII related D1 polypeptide was most abundant (bands 2 and 4). In contrast, CP43' in iron stressed cells was closely associated with the PSI characteristic PsaA/B heterodimer protein (bands 1 and 5). Moreover, it appears that most of the CP43' is closely attached to the monomeric form of PSI, which is more abundant in cells under iron limited conditions (Sandström et al., 2001a).

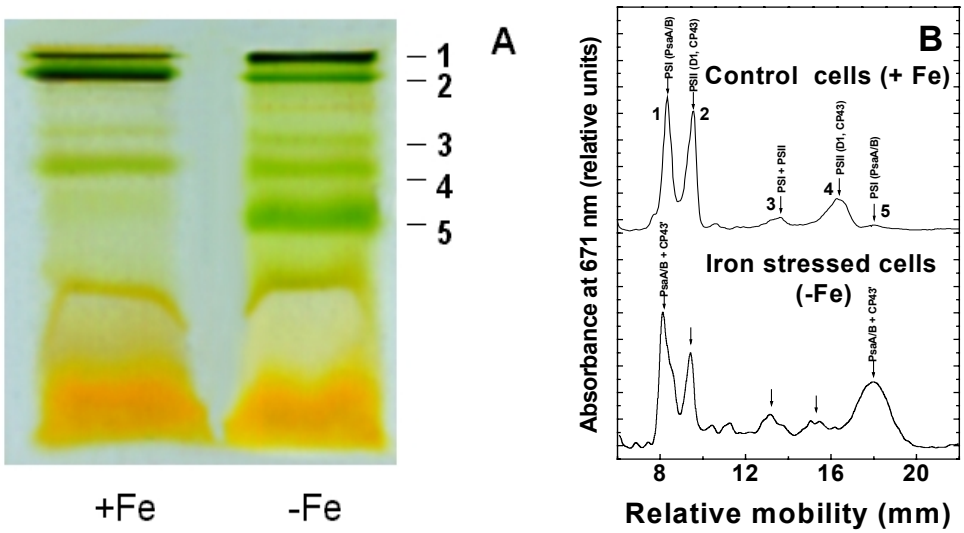

Fig. 1. A. Non-denaturating SDS-PAGE of thylakoid membranes isolated from control (right) and iron stresses (left) *Synechococcus* cells. B. Typical densitograms of non-denaturating SDS-PAGE profiles of chlorophyll-protein complexes in thylakoid membranes from control and iron stressed cells. All gels were loaded on an equal Chl basis of 20 μg lane^{-1} and the gels were scanned at 671 nm.

Sucrose gradient separation of solubilized thylakoids also resulted in several distinct bands. Iron sufficient cells gave the fractions containing mainly components of PSII, PSI and phycobilisomes with some CP43 (Fig. 2A, +Fe: fractions 1, 2 and 3 respectively). In iron deficient cells, CP43' had an opposite distribution compared to that of CP43 and was more abundant in the PSI fraction, thus supporting the green gels experiments. Plenty of the CP43' could also be found free on top of the gradient, forming an additional band in the 5% sucrose fraction (Fig. 2A, –Fe: fraction 1). The lowest fraction of iron deficient thylakoids (Fig. 2A, –Fe: fraction 4) also contained a considerable amount of CP43'. No detectable amount of any photosystem was found here. Fraction 4 had a chlorophyll absorption maximum at 670 nm, corresponding to that of free CP43' in fraction 1, and it still contained phycobilisomes as seen by the absorption spectrum (Fig. 2B, –Fe).

77K fluorescence emission spectra confirmed the composition of the iron sufficient fractions, showing typical maxima for PSII, PSI and the phycobilisome antenna (Fig. 2C, +Fe). In all fractions of the iron deficient cells, the phycobilisome emission at 650 nm gradually decreased and the emission at 685 nm increased when taking fractions from top to bottom (Fig. 2C, –Fe).

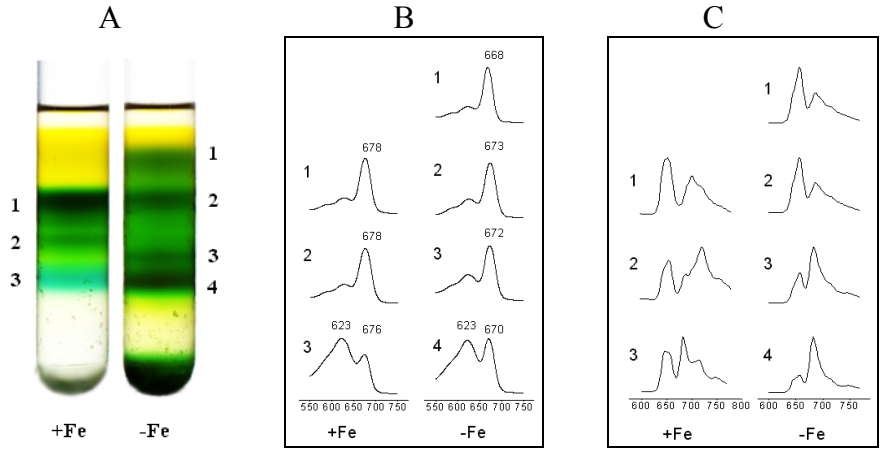

Fig. 2. Characterization of sucrose fractions. Thylakoids were solubilized in n-dodecyl β-D-maltoside and run on 0-50% step sucrose gradients overnight. A. Running profiles; B. Typical absorption spectra; C. Typical 77K fluorescence emission spectra (excitation to 560 nm). Fluorescence emission peaks centered at 650, 685, 695 and 715 nm are assigned to the phycobilisomes, Chl-protein antenna complexes (including CP43'), PSII and PSI core complexes respectively.

Discussion

It has previously been suggested that CP43' polypeptide does not contribute to the PSII light harvesting system (Falk et al., 1995). The separation of thylakoids of iron deficient *Synechococcus* sp. PCC 7942 over a native green gel system clearly demonstrates that CP43' associates to the bands containing the PsaA/B proteins of PSI rather than the D1/D2 proteins of PSII. The same tendency is observed when running the solubilized thylakoids on a sucrose gradient. This observation of CP43' being associated with the PSI core of iron deficient *Synechococcus* is in line with the recent finding that CP43' of iron deficient *Synechocystis* also associates to PSI (Bibby et al., 2001). Furthermore, they elegantly show that CP43' surrounds the PSI trimers as an antenna ring.

A high amount of CP43' runs free on top of the sucrose gradient, indicating that not all of this protein forms a stable association with the PSI core. The appearance of a PsaA/B immunoreaction in the 5th band of the green gel (Fig. 1), also points to the possibility of monomerization of some PSI complexes, as discussed before (Sandström et al., 2001b). Additionally, a fraction of CP43' is found on the sucrose gradients together with phycobilisomes, in the absence of any photosystem core proteins (Fig. 2A, fraction 4). The relative decrease of phycobilisomal fluorescence in this fraction suggests possible energy transfer from phycobilisomes to CP43'.

It has also been shown that the induction of CP43' is completed after 24 h of exposure to low iron, but the kinetics of the relative increase of the 77K fluorescence intensity at 685 nm is much longer and is fully completed after 96 h (Sandström et al., 2001b). It is therefore tempting to speculate that during the first 24 hours of CP43' induction under iron stress, this protein is being incorporated into the PSI antenna with high energy transfer capabilities, and contributes little to low-temperature

fluorescence. The later increase of the 685 nm fluorescence maximum of iron deficient *Synechococcus* sp. would then derive from accumulating CP43', less strongly associated with PSI.

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