S6-003

Dynamics of photosystem I: Ca^{2+}-based oligomerization and response to iron deficiency by induction of a new antenna built by IsiA

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Introduction

Oxygenic photosynthesis by cyanobacteria is an important source of global biomass production. This process is carried out by two photosystems acting together to generate ATP and reducing power. One of them, photosystem I (PSI), plays a central role in different energetization pathways, e.g. linear and cyclic electron transport. Whereas its structure has recently been determined at high resolution (Jordan et al., 2001) very little is known about its biogenesis (see S4-011, (Schwabe & Kruip, 2000)) and about its dynamic interaction with other proteins in the lipid bilayer, especially under different environmental conditions.

We present here two examples of the high flexibility of PSI in sensing and acting to its molecular environment: First, we describe a calcium-based switch which is used to sense the lumenal pH and to control the oligomerization status of PSI. Second, we describe the response of cyanobacteria to iron deficiency on a molecular level. Iron is the main limiting factor of cyanobacterial growth in aquatic environments. Cyanobacteria respond by the induction of special genes, like IsiA which is also called CP43´. Here we show that IsiA forms a ring around PS I and that it functions as a new light harvesting antenna system specific for PSI under iron limitation. This is the first report of a new membrane-integral antenna system in cyanobacteria and shows the flexibility of the photosynthetic electron transport chain (Boekema et al., 2001).

Materials and Methods

Strains of *Synechocystis sp.* PCC 6803 and *Synechococcus sp.* PCC 7942 were cultured in BG-11 medium with or without iron at moderate light intensities. Thylakoid membranes were isolated and used to purify PSI and PSI-IsiA complexes as described (Boekema et al., 2001; Wenk & Kruip, 2000): After solubilization by dodecylmaltoside, two consecutive HPLC steps, an anion-exchange chromatography (Poros HQ, Applied Biosystems) followed by a hydrophobic interaction chromatography (Poros Butyl, Applied Biosystems), yielded pure PSI and PSI-IsiA complexes. Size exclusion chromatography was done on a BioSilect 400 column (BioRad). For the calcium effect experiments, thylakoid membranes were incubated for 30

min in the indicated buffer, solubilized and immediately applied on an anion-exchange column (Poros HQ). The amount of monomeric and trimeric PSI could be calculated from the peak areas. For SDS-PAGE separation a Tris-Tricine system was used. De-novo sequencing of peptides was done on a quadrupole/time-of-flight hybrid mass spectrometer (Q-TOF2, Micromass). Electron microscopy of negatively stained particles was performed with a Philips CM10 electron microscope at 52000x magnification. Multi-reference alignment, multivariate statistical analysis and classification were performed with IMAGIC and Grip software. Flash-induced absorption changes were measured at 703 nm, the antenna size was calculated from the signal-amplitude using an extinction coefficient of 64 mM^{-1} cm^{-1}.

Results

PSI oligomerization - The monomer-to-trimer ratio in thylakoid membranes can be assessed by membrane solubilization followed by fast HPLC separation (Kruip et al., 1999). Fig.1 shows a typical elution profile of such a separation. The first peak eluting around 3 min represents monomeric, the second peak at 5 min trimeric PSI. When thylakoid membranes were depleted of calcium, mainly monomeric PSI (60-70%) was detected (Fig. 1, lower trace). In contrast, addition of calcium yields predominantly trimeric PSI (85-90 %, Fig. 1, upper trace). This effect was calcium specific, only strontium could replace calcium to some extent (data not shown). Analysis of the concentration dependence showed that calcium is effective in the micromolar range (data not shown).

In another series of experiments, the pH dependency of this calcium effect was investigated (Fig. 2). If the pH falls below a threshold value of around 5.5, even the presence of calcium is not enough to prevent monomerization. At a pH of 4.5 almost all PSI is in a monomeric form (Fig.2, lower trace).

By binding assays using radioactive Ca^{45} it could be shown that the PS I subunit PsaL specifically binds calcium (P. Chitnis, data not shown). We also tested other cyanobacteria for calcium-dependent PSI trimerization: All unicellular cyanobacteria tested so far (*Synechococcus elongatus*, *Mastigocladus laminosus*) were found to exhibit this effect.

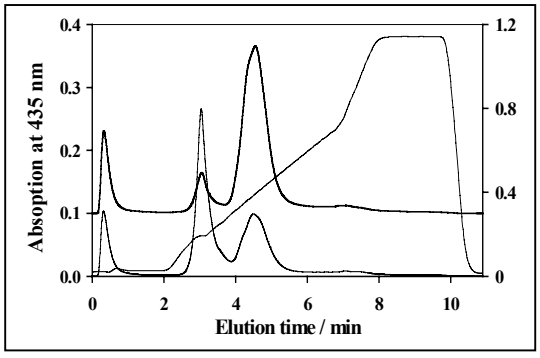


Fig.1 Trimerization is calcium-dependant. Elution profile of membrane extracts, either preincubated with 1 mM $CaCl_2$ (upper trace) or with 5 mM EGTA (lower trace). Separation was done on an anion-exchange column (Poros50, Applied Biosystems) using a magnesium sulphate gradient for elution.

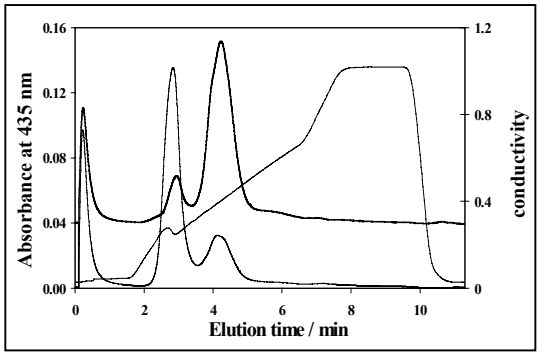


Fig.2 The Ca-effect is pH-dependant. HPLC analysis pattern (see Fig.1) of membranes in the presence of 1 mM calcium. Membranes were incubated prior to solubilization for 30 min at pH 6.5 (upper trace) or pH 4.5 (lower trace).

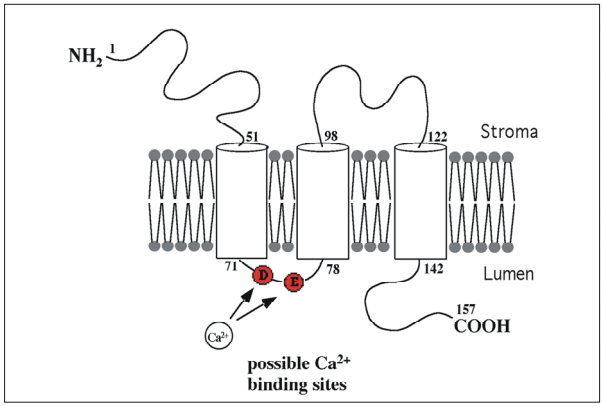


Fig.3 Folding topology of PsaL.
PsaL contains three predicted transmembrane helices which has been verified by a high resolution X-ray analysis (Jordan et al., 2001). The first lumenal loop contains two acidic residues (D73, E75 in *Synechocystis*) which could be part of a pH-sensing calcium binding site.

PsaL is known to be absolutely required for trimer formation (Chitnis & Chitnis, 1993). It forms the so-called connecting domain within the trimer (Jordan et al., 2001). Sequence alignments showed that *Synechocystis* as well as *Synechococcus elongatus* have only two acidic residues in the lumen which could serve as potential binding sites for calcium. From these data the following physiological model of trimerization can be drawn at the molecular level (Fig.4): Under low light conditions the lumenal pH is well above 5.5, the acidic residues D73 and E75 are charged and bind calcium thus allowing trimerization. Under these conditions, trimeric PSI abounds. Upon illumination photosynthetic electron transport leads to acidification of the thylakoid lumen. If the lumenal pH falls below 5.5, the acidic amino acids are protonated, calcium is released and monomerization is triggered, making monomeric PSI the favoured form.

We constructed a new *psaL* deletion mutant to support this model experimentally. As expected, the mutant was unable to form trimeric PSI (data not shown). In addition, a striking feature was its inability to grow photoautotrophically at low light conditions (< 5 µE) where wild type cells continue to grow (data not shown). Under normal light conditions the growth of both strains was indistinguishable. This clearly demonstrates that PSI exists under low light conditions in a trimeric form which is indispensable for cell growth under these conditions.

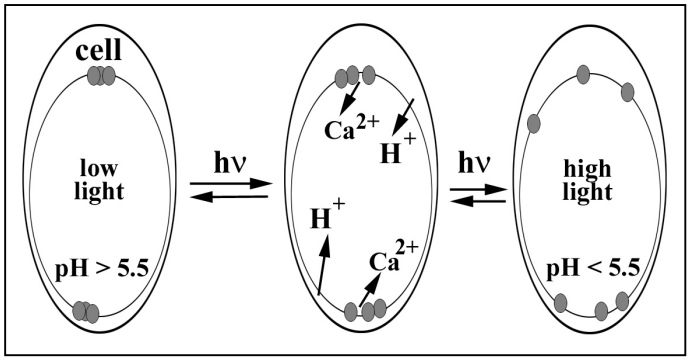


Fig. 4 Regulatory model of PSI trimerization.
Under low light conditions PSI exists mainly as a trimer hold together by calcium ions. Light leads to lumen acidification and calcium release from PSI which triggers mono-merization if the lumenal pH falls below 5.5.

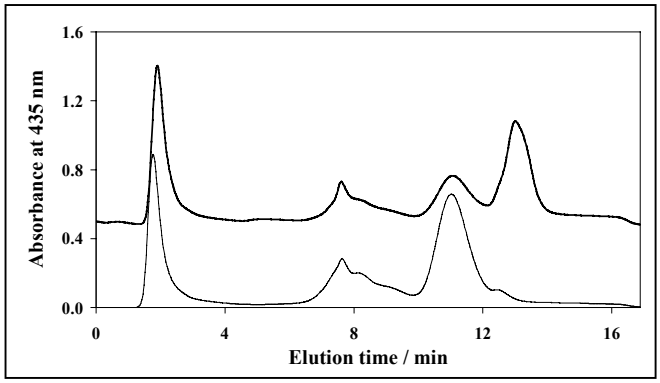
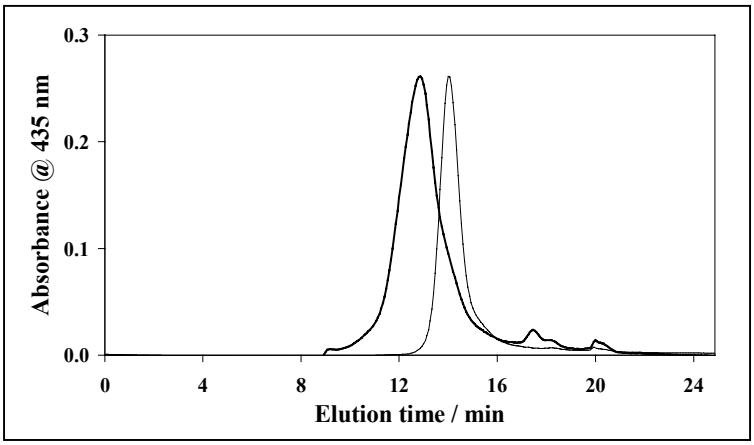


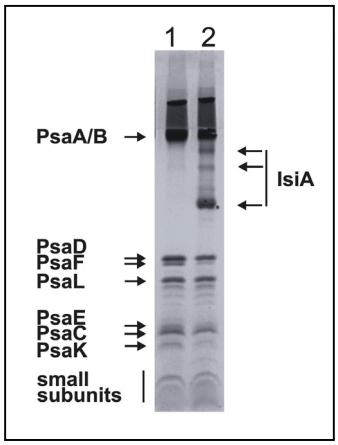
Fig.5 Iron deprivation induces formation of a new protein complex.
Elution profiles of extracts from thylakoid membranes of *Synechococcus* 7942 cells either grown with (lower trace) or without iron (upper trace). Separation was done by anion-exchange chromatography (Poros50) using a linear magnesium sulphate gradient for elution.

Response to iron deficiency - If *Synechococcus sp*. PCC7942 or *Synechocystis sp.* PCC6803 cells were grown for 4 days in BG-11 medium without iron, characteristic changes in the composition of protein complexes in the thylakoid membrane can be seen (Fig. 5). Anion exchange chromatography reveals the emergence of a new protein complex eluting around 13 min under iron limitation (Fig. 5).

At the same time the amount of trimeric PSI (eluting at 11 min) is reduced by 60-80% compared to cells grown under normal conditions. The new complex was purified to homogeneity by subsequent hydrophobic interaction chromatography (data not shown). Analytic size-exclusion chromatography gave an apparent molecular weight of 1550 kDa for the complex which is much larger than the 900 kDa determined for trimeric PSI (Fig.6). SDS-PAGE revealed that the iron-induced complex contains all PSI subunits together with additional bands at 36, 48 and 53 kDa (Fig. 6). All bands which could not be assigned were cut out and subjected to tryptic digestion followed by *de-novo* sequencing by ESI mass spectrometry. All three bands yielded the same pattern of tryptic peptides. Seven peptides (858.2844, 1128.4644, 1204.4044, 1360.4644, 1655.6843, 1695.6644, 2286.8167) were sequenced and resulted in perfect matches with IsiA, a known iron induced protein from *Synechococcus* 7942. Therefore, **the iron-induced complex was identified as PSI-IsiA complex** (Boekema et al., 2001).

IsiA shows strongest homologies to CP43, a chlorophyll-binding subunit of PSII, and hence was also called CP43´. The main difference is the lack of a large hydrophilic loop of about 100 amino acids. As all potential chlorophyll binding sites are conserved a similar pigment content can be assumed. This concurs with our Chl/P700 measurements which indicate a 60 % increase in antenna size for the PSI-IsiA complex (161 ± 20 Chl/P700) in comparison to trimeric PSI (103 ± 9 Chl/P700).





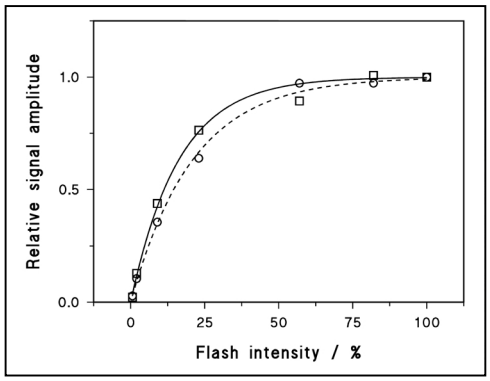


Fig. 7 IsiA functions as PSI antenna. Light saturation curves for trimeric PS I (circles) and for the PSI-IsiA complex (squares). The data were analyzed quantitatively by the equation (see text): $\Delta A=\Delta A_{max}$ x [1-exp(-*k* x rel. flash int.)].

Fig. 6 SDS-PAGE analyses of purified complexes. Proteins were separated by a Tris-Tricine gel system and stained with Coomassie. All proteins were identified as indicated.

If one assumes 12 chlorophyll molecules bound to IsiA and an antenna of 96 Chl for PSI, a theoretical antenna size of 168 Chl/P700 can be calculated for the PSI-IsiA complex, which fits our experimentally determined antenna size. The IsiA content also leads to a blue-shift of 4 nm in the absorption spectrum of the PSI-IsiA complex (675 nm) in comparison to PSI (679 nm). To test whether IsiA indeed functions as a functional antenna for PS I we measured light-saturation curves (Fig. 7). A quantitative treatment of the data revealed that the absorption cross-section for the PSI-IsiA complex is about 44 % higher compared to PSI (*k*, which is a measure of photon capture efficiency, is 0.045 ± 0.003 for PS I and 0.065 ± 0.007 for the PSI-IsiA complex). This indicates together with fluorescence spectra and PSI kinetics (data not shown), that IsiA is indeed a functional antenna for PSI.

Electron microscopy of the PSI-IsiA complex shows circular-shaped flat particles with a diameter of 345 Å (Fig. 8A). Single particle image analysis revealed a trimeric PSI which is surrounded by a ring of 18 densities (Fig. 8B-D). About 95 % of the particles were slightly tilted (Fig. 8C,D), only a limited number showed well preserved three-fold symmetry (Fig. 8B). A closer inspection showed that the particles face the carbon support with the bulky stromal surface explaining the numerous tilted particles. The ring must be attributed to 18 copies of IsiA. It can be further interpreted by comparison with the homologues CP43 subunit of PSII (Zouni et al., 2001). Its projected density is expected to have a very similar surface and has been modelled into the untilted projection (Fig. 8E). It fits into each of the 18 densities (black dots), if the periphery of the PSI-IsiA complex is corrected for an attached detergent shell.

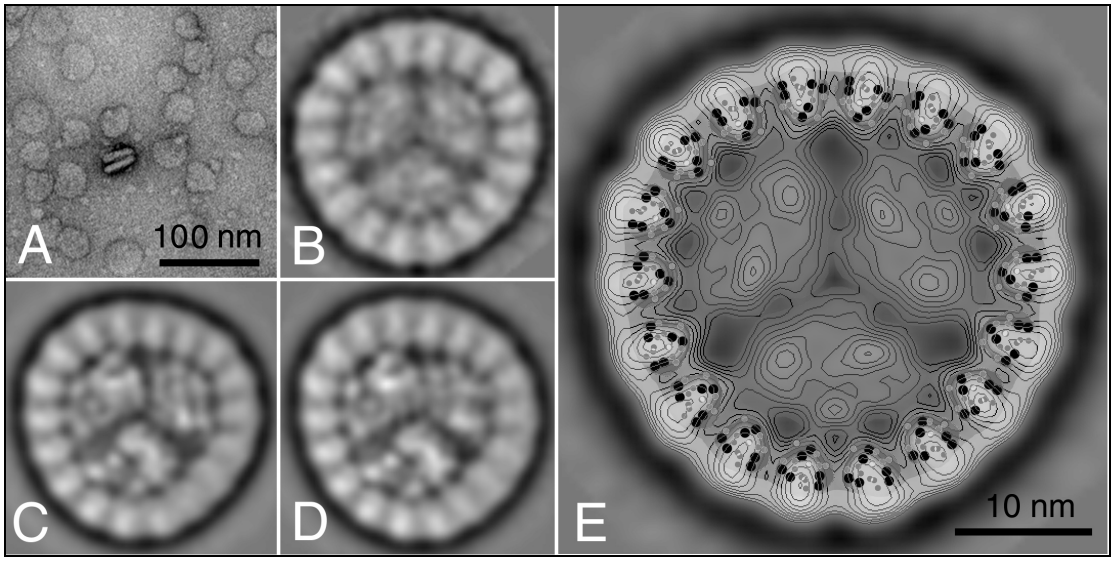


Fig.8 IsiA forms a ring around PSI. A, Typical electron micrograph of purified PSI-IsiA complexes. **B-D**, results of multivariate statistical analysis and classification of 5200 top-view projections; average images of 231, 2000 and 200 projections, respectively. **E**, contoured version of the three-fold symmetrized class-sum from B (see text for details)

Discussion

PSI oligomerization – We have shown that PSI contains a pH sensor which is based on calcium binding. Further we propose that the acidic residues D73 and/or E75 are part of the calcium binding site. Corresponding site directed mutants are in the segregation process and should answer this question soon. Indeed, in the high resolution structure of trimeric PSI (Jordan et al., 2001) a metal ion, presumable calcium, can be seen which is bound at the interface between monomers. Functional data indicate that trimeric PSI is necessary for

growth under low light conditions. This could be due to an increased absorption cross-section for photons. On the other hand monomeric PSI could be advantageous under high light conditions: Its higher diffusion coefficient would allow faster rearrangements of membrane protein architecture which must occur during the acclimation process.

Response to iron deficiency – We have shown that iron deficiency leads to remodelling of trimeric PSI: The iron-induced IsiA protein forms a ring of 18 subunits around existing trimeric PSI complexes. Each IsiA molecule contains chlorophyll and functions as additional antenna system for PSI. We could not find evidence for PSII-IsiA complexes. Therefore the molecular function of IsiA is different from its proposed roles as alternative antenna for PSII, as protection against high light or as chlorophyll-storage protein. Strikingly, the high-resolution PS I structure (Jordan et al., 2001) shows certain chlorophyll molecules at the PSI periphery which are ideally located for fast excitation transfer from the IsiA antenna to the PSI reaction centre.

Whereas most cyanobacteria contain only one copy of the *isiA* gene, oxychlorophytes contain a very similar chlorophyll-b binding protein encoded by the *pcb* genes. It will be interesting to learn, how the Pcb proteins are bound to reaction centers, especially since ecotypes that are adapted to low light environments contain multiple copies of these genes.

In summary, we have characterized a new antenna system for PSI which is induced only under certain environmental conditions. It resembles rings of LH-proteins found in purple bacteria. Together with LHC proteins from chloroplasts, we now know three types of membrane-integral antenna sytems all serving the same purpose but with different structure. They are perfect examples of convergent evolution.

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