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The discovery of an iron-stress-induced antenna ring around the photosystem I trimer in cyanobacteria.

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Introduction

The low abundance of iron in the open oceans has been demonstrated to be the limiting factor for photosynthetic activity and growth of phytoplankton (Martin *et al.* 1994 and Behrenfeld *et al.* 1996). Cyanobacteria, a major class of phytoplankton display an iron-stress-response to adapt to frequently occurring conditions of low iron. A characteristic feature of this response is the expression of the 'iron-stress-induced' gene, *isiA* (Straus 1994) the product of which, here called CP43' becomes the most abundant chlorophyll containing protein in the iron-stressed cyanobacterial cell (Burnap *et al*. 1993) although, its function remains unknown. CP43' shows significant homology with the CP43 chlorophyll *a*-binding protein of photosystem II (PSII). They share the same six transmembrane domains and are predicted to bind about the same level of chlorophyll *a*. However, CP43' lacks the large extrinsic loop consisting of about 150 amino acids, which joins the lumenal ends of helices 5 and 6 of CP43 (Burnap *et al.* 1993 and Falk *et al.* 1995). In this report we show that CP43' associates with photosystem I (PSI) to form a ring of 18 copies around a trimeric PSI reaction centre core and in so doing significantly increases the size of the light harvesting system of PSI. The utilisation of a PSII-like protein as an extra antenna for PSI emphasises the flexibility of cyanobacterial light harvesting systems and seems to be a strategy which compensates for the lowering of phycobilisome and PSI levels in response to iron deficiency.

Materials and methods

Synechocystis sp. PCC6803 with a Histidine-tag on CP47, the internal light harvesting antenna protein of PSII (Bricker *et al.* 1993) was grown photoheterotrophically in the presence and absence of iron (Bibby *et al.* 2001). Thylakoids were isolated and solubilised according to Tang and Diner (1994). The soluble fraction was subsequently subjected to Ni^{2+}- affinity chromatography. PSII was selectively bound to the column via the His-tag while the non-bound fraction (containing PSI) was loaded onto a 0.5 M sucrose density gradient. The biochemical characterisation of isolated PSI complexes from this gradient were conducted according to Bibby *et al*. (2001). Structural information of PSI complexes was obtained by electron microscopy of PSI particles stained with 2% uranyl acetate and subsequent single particle image analysis. Digitised electron micrographs provided single particle data sets of approximately 3000 (CP43'-PSI supercomplex) and 4000 (PSI trimer). All

subsequent processing was performed within the IMAGIC-5 software environment (van Heel *et al*. 1996, 2000). Co-ordinate data sets were obtained from the RCSB Data bank (www.rcsb.org) under the entry codes for 1C51 (PSI 4 Å structure, Krauß *et al.* 1996) and 1FE1 (PSII 3.8 Å structure, Zouni *et al.* 2001).

Results

Photosystem I fractions isolated from normal and iron-stressed cells were separated on sucrose density gradients (see methods). In the case of normal cells, the sucrose gradient separated two chlorophyll *a*-containing fractions, corresponding to PSI monomers and PSI trimers. In addition to these fractions, the iron-stressed cells produced two extra bands, one less dense than the PSI monomer and one more dense than the PSI trimer. SDS-PAGE (Fig. 1a) showed the less dense of the iron-stress-induced fractions to be free CP43' (confirmed by immunoblotting) and the more dense to be a CP43'-PSI complex consisting of the ~75 kDa PsaA/PsaB reaction centre subunits of PSI (PSI RC), the CP43' protein and other lower molecular weight PSI proteins. HPLC size exclusion chromatography gave an estimated molecular mass of approximately 1.9 MDa for the CP43'-PSI complex compared with 1 MDa for the PSI trimer. Optical absorption and 77 K emission spectra of PSI trimers, free CP43' and the CP43'-PSI complexes are shown in Fig. 1b. Although the absorption spectrum of the CP43'-PSI complex had a blue shifted, long wavelength absorption maximum compared with that of the PSI trimer due to the presence of CP43', the 77 K fluorescence spectrum of the CP43'-PSI complex gave the same maximum as that of the PSI trimer alone. Since isolated CP43' was highly fluorescent with a maximum at 685 nm, then it is concluded that in the CP43'-PSI complex, CP43' is associated with PSI in such a way as to allow efficient energy transfer. Excitation spectra for the 720 nm emission from the CP43'-PSI complex confirmed that the majority of the CP43' present efficiently transferred energy to PSI. To gain a better understanding of the association of CP43' with PSI, single particle analysis of the CP43'-PSI complex was conducted on images obtained by electron microscopy Fig 2.

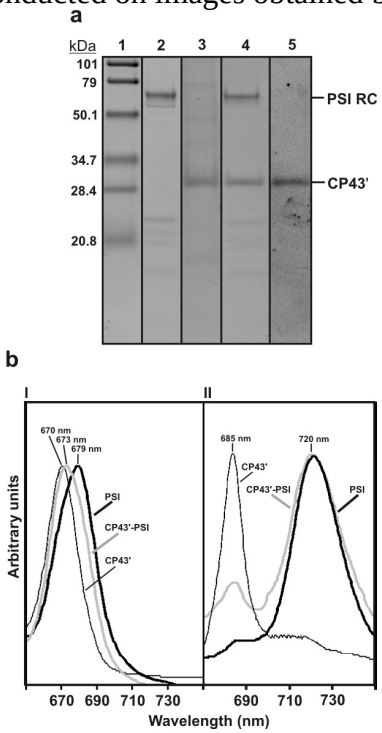

Figure 1 (a) SDS-PAGE of fractions derived from sucrose density centrifugation. Lane 1, molecular markers; Lane 2, PSI trimers; Lane 3, CP43'; Lane 4, CP43'-PSI complex and Lane 5, Western blot using antibody to CP43' kindly provided by L. Sherman, Purdue University, Indiana, USA. PSI RC corresponds to the PsaA and PsaB reaction centre (RC) proteins of PSI. **(b)** Optical absorption and fluorescence properties of fractions derived from sucrose density centrifugation. **I** Room temperature absorption spectra. **II** Fluorescence emission spectra at 77 K excited by 440 nm light. Spectra are normalised at their peaks to aid comparison.

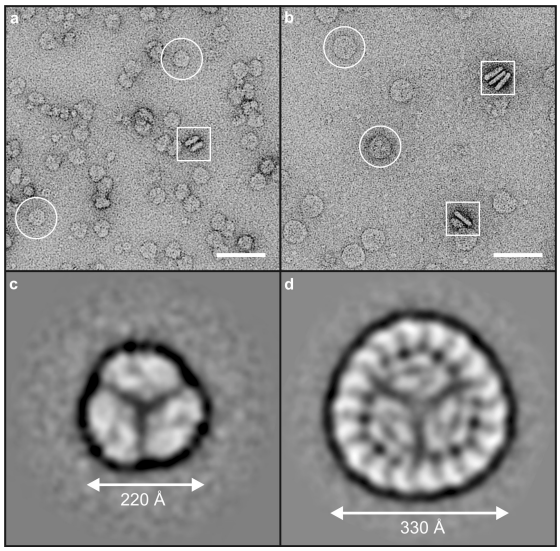

Figure 2 Typical electron micrographs of PSI trimers **(a)** and CP43'-PSI **(b)** complex in negative stain. The bar in both a and b represents 50nm. Most complexes are top views, probably of the stromal surface (ringed), however, occasional side views were observed (boxed). Image processed top views of negatively stained PSI trimers **(c)** and CP43'-PSI complexes **(d)**. Of particular note is that the iron-stressed-induced complex contains a trimer of PSI surrounded by a 55Å thick ring of density corresponding to 18 copies of CP43'

Top view averages of the PSI trimers and the CP43'-PSI complexes are shown in Fig. 2c and 2d at a resolution of about 25 Å. The thickness of the PSI trimer and CP43'-PSI trimer supercomplex is approximately the same, as seen in Fig. 2a and 2b (boxed), and estimated to be about 90 Å. This indicates that the additional ring of CP43' in the CP43'-PSI complex is contained within the membrane.

Discussion

A 4 Å X-ray structure of the cyanobacterial PSI trimer has been published (Krauß *et al.* 1996). Moreover, there is a 3.8 Å structural model of PSII isolated from *S. elongatus* (Zouni A *et al.* 2001) which, as suggested from electron crystallography (Hankamer *et al.* 1999), shows CP43 to be a ring of three pairs of transmembrane helices. In Fig. 3 we have modelled the X-ray structures of the PSI trimer and CP43 into the projection map of the CP43'-PSI trimer supercomplex. Given that CP43 binds at least 12 chlorophyll molecules within its six helical bundle (Zouni et al. 2001), we conclude that the additional antenna size of the iron-stress-induced CP43'-PSI supercomplex is approximately 216 chlorophyll *a* molecules. Since the PSI trimer contains about 300 chlorophyll molecules (Schubert *et al.* 1997) then the light harvesting ability of the supercomplex has increased by approximately 72% compared with that of the normal trimer. This increase in antenna size is almost certainly in response to the reduction in the level of the light harvesting phycobilisomes and PSI complexes due to the heavy demand on iron for their synthesis and assembly (Straus 1994). Among the various hypotheses for the physiological role of CP43', it has been suggested that it may act as an additional light harvesting system for PSII given that CP43' is a PSII-like protein (Strauss 1994). Our discovery of its functional association with PSI is therefore somewhat surprising, although the formation of an antenna ring around the PSI trimer does provide a functional significance for the trimeric nature of PSI. The formation of a ring of light harvesting chlorophyll binding proteins around a reaction centre is reminiscent of the organisation of the antenna systems of anoxygenic purple photosynthetic bacteria (McDermott *et al.* 1995 and Cogdell *et al.* 1999). We can now conclude for the first time that similar rings can occur in oxygenic photosynthetic organisms. Moreover, our discovery has implications for understanding the light harvesting systems of green oxyphotobacteria, formally known as Prochlorophytes. These organisms contain *pcb* genes which encode chlorophyll *a*/*b* binding

proteins. Gene sequencing has shown that the *pcb* genes are homologous to the *isiA* gene of cyanobacteria (LaRoche *et al.* 1996). Therefore it is highly likely that similar antenna rings exist in this class of organism, see Barber *et al.* this symposium.

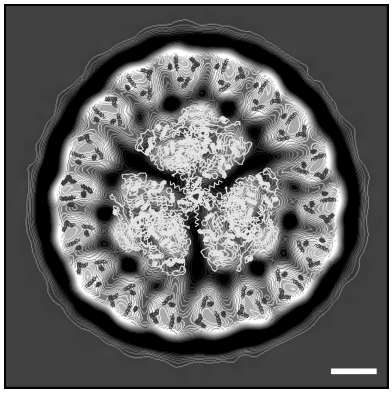

Figure 3. The PSI trimer structure and CP43 helix organisation derived from X-ray crystallography overlaid onto the projection map of the CP43'-PSI. The bar represents 5nm. Good correlation between the X-ray and electron microscopy data confirms the presence of a PSI trimer within the centre of an 18-member ring of CP43'

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