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Three-dimensional model and characterisation of the iron-stress induced CP43'-Photosystem I supercomplex isolated from the cyanobacterium *Synechocystis* PCC 6803

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Introduction

Although iron is the fourth most abundant metal in the Earth's crust in most aquatic ecosystems it can be sufficiently low to limit photosynthetic activity. This is mainly due to the low solubility of Fe^{3+} above neutral pH in oxygenic ecosystems. As a result, cyanobacteria and other microorganisms have evolved a number of responses to cope with frequently occurring conditions of iron deficiency reviewed in (Straus 1994). One such response is to express two 'iron-stress-induced' genes, *isiA* and *isiB*, which are located on the same operon. The *isiB* gene encodes for flavodoxin, which can functionally replace the iron containing ferredoxin. The *isiA* gene encodes for a protein often called CP43' because it has an amino acid sequence homologous to that of the chlorophyll *a*-binding protein, CP43 of photosystem II (PSII). Like CP43, CP43' is predicted to have six transmembrane helices and, judged on conservation of histidine residues, is likely to bind the same number of chlorophyll *a* molecules. The major difference is that CP43' lacks the large hydrophilic loop which joins the lumenal ends of helices V and VI of CP43.

Although the discovery of the CP43-like iron-stress-induced protein was made some time ago (Guikema & Sherman 1984) its precise function has not been elucidated. There have been at least four postulates: (i) CP43' aids recovery of cells by acting as a chlorophyll store so that PSII and photosystem I (PSI) complexes can be quickly synthesised when iron becomes readily available in the environment. (ii) CP43' protects PSII from photoinduced damage by acting as a dissipater of excitation energy. (iii) CP43' is a functional replacement for CP43 in PSII during iron-starvation. (iv) CP43' acts as a light-harvesting complex under iron-stress conditions, mainly for PSII but perhaps also for PSI.

Recently we showed for the first time that a CP43'-PSI trimer supercomplex can be isolated from the cyanobacteria *Synechocystis* PCC 6803 when grown under iron-stressed conditions (Bibby, Nield, Barber 2001). Here we report a more detailed description of this supercomplex by presenting a preliminary 3D model of its structure.

Materials and methods

Growth conditions - All studies were conducted on preparations isolated from *Synechocystis* sp. PCC 6803 having a histidine tag attached to the C-terminus of the PSII protein, CP47 (Bricker *et al*.1998). Iron-stressed cultures were obtained by growing cells in BG-11 medium but lacking iron-containing compounds. Cultures were harvested after 3 days and in the case

of iron starvation the cells had a blue shift in their long wavelength absorption band of about 7 nm compared with normal cells. Thylakoid membranes were isolated and solubilized with 1% β-D-dodecyl maltoside at 4^{o}C for 10 min and centrifuged at 45 000 rpm using a Beckman Ti70 rotor. The supernatant was then passed through a Ni^{2+}-affinity column to remove PSII and the non-bound fraction containing PSI was collected. The PSI enriched fraction was subjected to sucrose density gradient centrifugation and the resulting bands were independently removed for biochemical and structural characterization.

Biochemical characterisation - SDS-PAGE and Western blotting were performed as described in Hankamer *et al*. (1997). Optical absorption spectra were measured using a Shimadzu MPS 2000 spectrometer. Steady-state fluorescence spectra were obtained using a Perkin Elmer LS50 at 77 K and measured with an excitation wavelength of 440 nm.

Electron microscopy and image processing - Preparations were negatively stained with 2% uranyl acetate on glow discharged carbon evaporated grids and imaged using a Philips CM 100 electron microscope at 80 kV. Electron micrographs were digitized using a Leafscan 45 densitometer set at a step size of 10 microns. Single particle data sets were analysed by interactively selecting all possible particles from the micrographs using the IMAGIC-5 software environment (van Heel *et al*. 2000). The relative orientations of the improved class averages were determined by the angular reconstitution technique (van Heel 1987), allowing for an initial 3D reconstruction to be obtained by exact-back projection.

Molecular modelling - Co-ordinate data sets were obtained from the RCSB Data Bank (www.rcsb.org) for the entry codes 1C51 PSI 4 Å structure (Krauß *et al.* 1996) and 1FE1 PSII 3.8 Å structure (Zouni *et al*. 2001). These structural models were visualized using the program Swiss-PDB viewer (Guex & Peitsch 1997) and modeled into the calculated 3D map using the 'O' modeling software package (Jones *et al*. 1991).

Results

Sucrose gradient density centrifugation and size exclusion HPLC analysis (see Fig. 1) of the solubilized PSI fractions indicated that the two PSI bands obtained with normal cells corresponded to the approximate molecular masses expected for a monomeric (~356 kDa) and trimeric (1068 kDa) PSI complex (Jordan *et al*. 2001) with the trimer being the dominant species. The additional peaks observed after iron-stress correspond to native chlorophyll-binding CP43' (~47 kDa) and a high molecular weight chlorophyll-containing species of about 1900 kDa indicative of a supercomplex composed of CP43' and PSI.

Spectral characterisation - The room temperature optical absorption spectra of the isolated PSI trimer, CP43' and the CP43'-PSI supercomplex showed that they had long wavelength absorption maximum at 680, 670 and 673 nm respectively. Fluorescence measured at 77 K showed that the PSI trimer had an emission maximum at 720 nm while CP43' fluoresced maximally at 685 nm. However, in the case of a CP43'-PSI supercomplex the emission profile was similar to that of PSI except for some weak emission at about 685 nm. On addition of 0.1% Triton X-100, this weak signal at 685 nm changed to the dominant emission indicating that the detergent had uncoupled CP43' from PSI and therefore suggests that in the untreated sample, energy is efficiently transferred from CP43' to PSI. Sucrose density gradient analyses showed that indeed the Triton X-100 treatment converted the CP43'-PSI band into trimeric PSI and free CP43'. Further confirmation that CP43' within the CP43'-PSI supercomplex was functionally coupled to PSI was made by measuring excitation spectra for 77 K fluorescence emission at 720 nm.

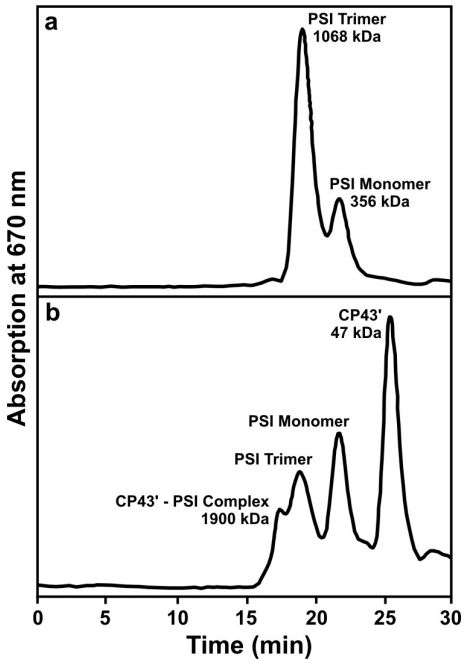

Fig. 1. HPLC size-exclusion chromatography of PSI-enriched fractions of *Synechocystis* isolated from (a) normally grown cells and (b) iron-stressed cells, detected at 670 nm for chlorophyll-containing proteins.

Structure of the CP43' –PSI supercomplex - We have shown previously by electron microscopy and single particle analysis that the CP43'-PSI supercomplex is composed of a PSI trimer surrounded by a ring of 18 subunits of CP43' (Bibby *et al*. 2001). The structural model presented in this report was obtained by analysing top views only but other views were also observed in the electron micrographs, including side elevations and views attributed to tilting particles. We have therefore taken advantage of these other views to obtain a range of class averages of the supercomplex and a 3D model has been calculated. Figure 2a shows nine typical class averages, taken from 76 class averages showing a range of orientations as derived from a 3000 particle data set. All 76 class averages were used to construct the 3D model. This 3D model is shown in Fig. 2b as surface rendered views at the same orientations as the class averages given in Fig. 2a. It clearly indicates that the central PSI trimer is surrounded by 18 CP43' subunits. According to Fourier shell correlation analysis (Fig. 2c) the 3D model has a resolution of about 24 Å.

Although the present 3D model of the CP43'-PSI supercomplex has potential for further refinement by electron cryo microscopy of non-stained vitrified samples, it does provide a framework in which to model the structures of PSI (Krauß *et al.* 1996) and CP43 (Zouni *et al*. 2001) obtained by X-ray crystallography of complexes isolated from the thermophilic cyanobacteria *Synechococcus elongatus*. The structure of the PSI trimer is now at a resolution of 2.5 Å (Jordan *et al.* 2001) but at present only the 4 Å model of Krauß *et al*. (1996) is available in the database. In the case of CP43 the 3.8 Å data is available (Zouni *et al*. 2001) and can be used to model the 6 transmembrane helices of CP43' and therefore the positioning of the tetrapyrrole headgroups of chlorophyll *a* (Fig. 3).

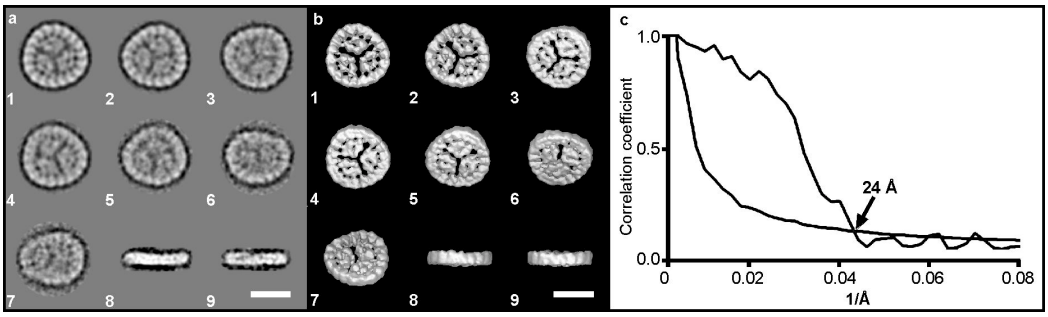

Fig. 2 Image processing of various 2D views of the CP43'-PSI supercomplex. (a) Selection of nine typical class averages taken from 76 different class averages used for the 3D reconstruction. (b) Surface rendered views of the final 3D map calculated by angular reconstitution viewed from the same angles presented in (a). (c) Fourier-shell correlation at $3\sigma\sqrt{3}$ gives a resolution of 24 Å for the 3D model. Bars represent 20 nm.

Discussion

We have isolated for the first time a cyanobacterial iron-stress-induced CP43'-PSI supercomplex (Bibby, Nield, Barber 2001) having an estimated molecular mass of about 1900 kDa. This mass is consistent with that predicted by calculation for a PSI trimer (1068 kDa) plus 18 copies of the CP43' protein (846 kDa).

Assuming each CP43' subunit binds at least 12 chlorophylls as does CP43 (Zouni *et al*. 2001), then the CP43'-PSI supercomplex would contain 216 or more chlorophylls. It is for this reason that the optical absorption spectrum of this supercomplex is significantly different to that of the PSI trimer alone. The chlorophyll *a* molecules bound within the CP43' protein have a long wavelength absorption maximum at about 670 nm. Therefore the long wavelength absorption peak shifts from 680 nm for PSI to 673 nm for the CP43'-PSI supercomplex. Some free CP43' in the supercomplex preparation could also contribute to this blue shift but fluorescence measurements suggest that this contamination is not significant. When isolated, the CP43' protein has a relatively high fluorescence yield at 77 K peaking at 685 nm. Although some emission at this wavelength was detected from the CP43'-PSI supercomplex the PSI low temperature emission at 720 nm dominated. Only after addition of 0.1% Triton X-100 to dissociate the CP43' protein from the PSI trimer was a large fluorescence emission seen at 685 nm from the supercomplex. We conclude therefore that the chlorophylls within the CP43' ring are excitonically coupled to those within the PSI trimer core. Given that the PSI trimer binds almost 300 chlorophyll *a* molecules (Jordan *et al*. 2001) we can conclude that the additional 216 chlorophylls in the CP43' ring increases the light harvesting capacity of the PSI reaction centres within the supercomplex by at least 70%.

The 3D model of the CP43'-PSI supercomplex presented in Fig. 2b was constructed using a number of top, intermediate and side views showing that the supercomplex has a diameter of about 330 Å and a thickness of approximately 80 Å in negative stain. Since the hydrophobic surfaces of the supercomplex must have a detergent layer the true diameter is likely to be slightly less. The 2D class averages (Fig. 2a) and 3D reconstruction (Fig. 2b) reveal rather flat stromal and lumenal surfaces, which is not expected for the stromal surface since the PSI trimer normally binds extrinsic PsaC, PsaD and PsaE proteins. However, we are confident that the 3D model is composed of characteristic stromal and lumenal views since differences can be

observed in the internal density distribution of the PSI monomers within different 2D class averages indicative of different orientations on the carbon grid (see Fig. 2a). There is a possibility that the uranyl acetate staining procedure used prior to imaging in the electron microscope dislodges the extrinsic proteins.

The modeling of the CP43‘-PSI supercomplex using the X-ray structures of the PSI trimer and of the PSII CP43 can provide a framework to start to understand how the chlorophylls of the CP43‘ ring transfer energy to those bound within the PSI trimer. In Fig. 3 we have modeled the position of the chlorophylls derived from the X-ray crystallography into the CP43‘-PSI complex. For convenience we have assumed that the transmembrane helix and chlorophyll organization is the same in CP43‘ and CP43 and that helices V and VI of CP43‘ are located closest to the reaction centre core as they are in the case of CP43 within the PSII structure (Hankamer *et al*. 2001). The resulting model is consistent with our finding that the chlorophyll molecules of the CP43‘ ring and PSI trimer are sufficiently close to facilitate energy transfer to the PSI reaction centres. Of particular note is that there seem to be possible entry points for energy transfer (starred in Fig. 3) corresponding to chlorophyll interdistances of 12 to 18 Å. The three entry points for each monomer seem to involve chlorophyll *a* molecules clustered close to helices c and d of the PsaB (single star) and PsaA proteins (three stars) and those probably associated with the PsaJ protein (double star) (Jordan *et al*. 2001). However this modeling is preliminary and will be improved with a better resolution of the 3D map of the CP43‘-PSI supercomplex and by having access to the co-ordinates of the 2.5 Å X-ray model of PSI.

The results presented here are not only important for understanding how certain cyanobacteria respond to iron-stress but also have implications with regard to the organization of light harvesting systems of the chlorophyll *a*/*b* containing oxyphotobacteria, formerly known as prochlorophytes. These green, oxygenic prokaryotes contain *pcb* genes that encode for chlorophyll *a*/*b* binding proteins similar to the IsiA/CP43' protein. Although there is usually only one *isiA* gene in phycobilisome-containing cyanobacteria, the number of *pcb* genes differ between the three known classes of prochlorophytes, *Prochloron*, *Prochlorothrix* and *Prochlorococcus* and even between different strains of *Prochlorococcus* (La Roche *et al*. 1996, Garczarek *et al*. 2000). It seems highly likely that one or more of these genes will encode chlorophyll *a*/*b* binding proteins which form a Pcb-PSI structure similar to that described here for the CP43'-PSI supercomplex. Indeed, such a supercomplex has recently been discovered in *Prochlorococcus*, strain SS120 (Bibby *et al*. 2001).

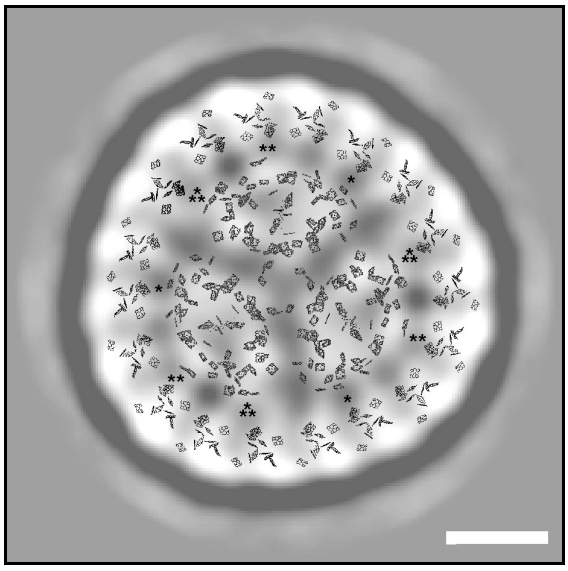

Fig. 3. Preliminary model of chlorophyll organisation of the CP43'-PSI supercomplex using co-ordinates from the 4 Å X-ray PSI trimer structure (Krauß *et al.* 1996) and 3.8 Å PSII CP43 structure (Zouni *et al.* 2001). The model is based on the co-ordinate datasets IC51 (PSI) and IFE1 (PSII). The modeled chlorophylls of CP43' come closest to those of PSI at the regions marked with stars. Bar represents 5 nm.

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References

Bibby TS, Nield J, Barber J (2001) *Nature* in press

Bibby TS, Nield J, Partensky F, Barber J (2001) *Nature* in press

Bricker TM, Morvant J, Marsi N, Sutton HM, Frankel LK (1998) *Biochim. Biophys. Acta.* **1409**, 50-57

Garczarek L, Hess W, Holtzendorff J, van der Staay G, Partensky F (2000) *Proc. Natl. Acad. Sci. USA* **97**, 4098-4101

Guex N, Peitsch MC (1997) *Electrophoresis* **18,** 2714-2723

Guikema JA, Sherman LA (1984) *Plant Physiol*. **74**, 90-95

Hankamer B, Morris EP, Nield J, Gerle C, Barber J (2001) *J. Struct. Biol*. in press

Hankamer B, Nield J, Zheleva D, Boekema EJ, Jansson S, Barber J (1997) *Eur. J. Biochem.* **243**, 422-429

Jones TA, Zou JY, Cowan SW, Kjeldgaard M (1991) *Acta Crystallogr. A* **47**, 110-119

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauβ N (2001) *Nature* **411,** 909-917

Krauß N *et al.* (1996) *Nature Struct. Biol.* **3,** 965-973
La Roche J *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**, 15244-15248
Straus NA (1994) in Molecular Biology of Cyanobacteria (ed. Bryant DA) pp 731-750, Kluwer Academic Press, Dordrecht, The Netherlands
van Heel M *et al*. (2000) *Quarterly Revs. Biophys.* **33**, 307-369
van Heel M (1987) *Ultramicroscopy* **21,** 111-124
Zouni A, Witt HT, Kern J, Fromme P, Kraus N, Saenger W, Orth P (2001) *Nature* **409**, 739-743