Spectroscopic study of CP43' complex and PSI-CP43' supercomplex of the cyanobacterium *Synechocystis* PCC 6803

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Abstract

The PSI-CP43' supercomplex of the cyanobacterium *Synechocystis* PCC 6803, grown under iron-starvation conditions, consists of a trimeric core Photosystem I (PSI) complex and an outer ring of 18 CP43' light-harvesting complexes. We have investigated the electronic structure and excitation energy transfer (EET) pathways within the CP43' (also known as the *isiA* gene product) ring using low-temperature absorption, fluorescence, fluorescence excitation, and hole-burning (HB) spectroscopies. Analysis of the absorption spectra of PSI, CP43', and PSI-CP43' complexes suggests that there are 13 chlorophylls (Chls) per CP43' monomer, *i.e.*, a number that was observed in the CP43 complex of Photosystem II (PSII) (Umena, Y. *et al. Nature* **2011**, 473, 55-60). This is in contrast with the recent modeling studies of Zhang, Y. *et al. (Biochim. Biophys. Acta* **2010**, 1797, 457-465), which suggested that IsiA likely contains 15 Chls. Modeling studies of various optical spectra of the CP43' ring using the uncorrelated EET model (Zazubovich, V.; Jankowiak, R. *J. Lumin.* **2007**, 127, 245-250), suggest that CP43' monomers (in analogy to the CP43 complexes of PSII core) also possess two quasi-degenerate low-energy states, A' and B'. The site distribution functions of state A' and B' maxima/full width at half maximum (FWHM) are at 684 nm/180 cm⁻¹ and 683 nm/80 cm⁻¹, respectively. Our analysis shows that pigments mostly contributing to the lowest-energy A' and B' states must be located on the side of the CP43' complex facing the PSI core, a finding that contradicts the model of Zhang *et al.*, but is in agreement with the model suggested by Nield *et al. (Biochemistry* **2003**, 42, 3180-3188). We demonstrate that the A'-A' and B'-B' EET between different monomers is possible, though with a slower rate than intra-monomer A'-B' and/or B'-A' energy transfer.

Keywords: Hole Burning, Fluorescence Excitation, Excitation Energy Transfer

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1. INTRODUCTION

Cyanobacteria account for more than half of the total photosynthetic productivity on Earth, and can be found in almost every conceivable environment, including most marine and freshwater habitats.¹ One of the main reasons that cyanobacteria have become so ubiquitous is that their strong and effective regulatory mechanisms maintain high photosynthetic production levels under various conditions of illumination or nutrient stress.² Their adaptation to low-iron environments is an important example. When grown under iron-deficient conditions, the cyanobacterial phycobiliprotein content and Photosystem I (PSI) to Photosystem II (PSII) ratio are reduced. To compensate for this, some cyanobacteria express the *isiA* gene product. Multiple copies of chlorophyll a (Chl a) binding IsiA protein surround the PSI trimer to compensate for PSI content decreases under the iron-stress conditions.³⁻⁵ This protein is a genetic homologue of PsbC, the CP43 protein of the PSII core, associated with the water-splitting and oxygen-evolving enzyme of photosynthesis. Therefore, it is often called CP43'.⁴⁻⁶

Although CP43 and CP43' have high amino acid sequence homology, they show some key differences. Indeed, in addition to different mass due mainly to a marked shortening of the large E loop on the luminal side of CP43', the His ligand that binds Chl 46 in CP43 is replaced with Gln in CP43' of *Synechocystis* PCC 6803, and a Met residue, which has been implicated as a possible ligand in Chl 37 of CP43, is converted to a Phe in CP43'.⁷ Both of these Chls have been suggested to play important roles in the excitation energy transfer (EET) dynamics of CP43, so comparison of the EET properties of these two protein complexes is of great interest.

A breakthrough in understanding the association of the proteins belonging to the core antenna family within the PSI core occurred a decade ago when two laboratories, using electron microscopy, discovered simultaneously that a supercomplex of trimeric PSI is encircled by a ring

of 18 IsiA proteins in two different cyanobacterial species (*Synechocystis* PCC 6803^{3,7} and *Synechococcus* PCC 7942⁸) when grown under limiting iron conditions. It has been established that while the whole PSI-CP43' supercomplex has a diameter of 320 Å and a thickness of 90 Å, each of the 18 CP43' monomer has a width of about 24 Å and a 20° inclination relative to its neighbors.^{9,10} Since then, much work has been dedicated to unraveling the spectroscopic properties and structure-function relationships in these complexes.^{4,5,11-29}

The addition of the 18-mer CP43' ring to PSI is an evolutionary adaptation to the reduction of the amounts of PSI and phycobilisomes under low-iron conditions, and it increases the light-harvesting efficiency of the PSI complexes by at least 70%.^{3,4,7,8} Many studies using different spectroscopic methods have revealed that the CP43' ring can transfer energy very effectively to the PSI core.^{4,5,7,8} In addition, some groups have suggested that photoprotection of PSI (though PSI is not particularly prone to photodamage) and PSII under unfavorable environmental conditions might be the main function of CP43' complex^{18,21}. Although significant progress has been made in our understanding of these complexes, many open issues remain. For example, the number of Chls constituting a CP43' monomer is still under debate. It is most often assumed to be the same as the number of pigments present in the CP43 complex. However, the latter number has varied over time.³⁰⁻³² For example, in 2001, the 3.8 Å resolution X-ray structure of a cyanobacterial PSII core³⁰ revealed the presence of 12 Chl *a* molecules per CP43 monomer. In 2004, the crystal structure of a cyanobacterial PSII core complex at 3.5 Å resolution³¹ suggested that each monomer has about 14 Chls. However, more recent structural data obtained at 3.0 Å³², 2.9 Å³³, and 1.9 Å³⁴ resolutions, respectively, established that the number of Chls is 13. In contrast, Ref.⁵ suggested 16-17 Chls per CP43' monomer in another cyanobacterium (*Synechococcus* PCC 7942), while the modeling studies discussed in Ref.¹⁰

proposed that each IsiA in the PSI-CP43' supercomplex from the *Synechocystis* PCC 6803 binds 15 Chls. The latter value was obtained from calculations of optimal energy transfer within the PSI-CP43' supercomplex.

In the present study, we show that each monomer of CP43' complex most likely possesses 13 Chls, in agreement with the number of pigments in CP43 of PSII,^{32,34} and energy transfer between quasi-degenerate states with uncorrelated site distribution functions (successfully applied previously to explain the various optical spectra of CP43 of PSII^{6,35}) can be used to explain the electronic structure and EET in the ring of CP43' complexes. Regarding the structure of the PSI-CP43' supercomplex (*i.e.*, the orientation of CP43' monomers and the PSI trimer in relationship to each other), we argue below that our data are consistent with the model suggested by Nield *et al.*⁹ and not with the model proposed recently by Zhang *et al.*¹⁰

2. EXPERIMENTAL SECTION

2.1. Sample isolation procedure. All glassware used for cell-growth was first washed with dilute HCl (0.01 times commercial, concentrated HCl) to eliminate any iron adhering to the glass. A pre-culture of cyanobacteria at an optical density of about 1.0 (at 600 nm) was grown for two days in BG-11 mineral medium, lacking iron-containing compounds, and then it was diluted 15 times in the same medium to keep the culture at an optical density of 0.1 (at 600 nm). The cultures then were grown for 21 days and harvested by centrifugation at $13800 \times g$ for 15 min at 4°C. For the isolation of CP43' complex, *Synechocystis* *psaFJ*-depleted mutant cells³⁶ were used (this mutant synthesizes much lower amounts of PSI, thus reducing the risk of PSI contamination). Approximately 15 g cells (wet weight) were suspended in 50 mM MES-NaOH, pH 6.0, 25% (w/v) glycerol, 5 mM CaCl₂, and 5 mM MgCl₂ at a ratio of 10 mL of buffer per 1 g

of cyanobacteria. The suspension was then centrifuged at $13800 \times g$ for 15 min at 4°C , the pellet was suspended in the same original buffer volume with 1 mM PMFS (Sigma) and $50 \mu\text{M/L}$ DNAase (Roche Diagnostics, Mannheim, Germany) added. The cells were then broken by sonication (Ultrasonic Processor XL2020, Heat System) for 45 min. Cycles of 1 min and 30 s sonication and 1 min storage of the tubes on ice were employed to avoid sample overheating. The resultant cell extract was clarified by centrifugation at $121 \times g$ for 5 min at 4°C to eliminate any cell debris and large particles. The supernatant was finally centrifuged at $13900 \times g$ for 35 min at 4°C , and the pellet contained thylakoid membranes.

2.2. Isolation of CP43' complex. The above pellet was incubated with the same buffer supplemented with 1 M guanidinium chloride (GuCl) for 30 min at 4°C , with gentle stirring to eliminate most of the phycobillisomes that contaminate the sample. The incubation was followed by a second centrifugation, and the resultant pellet was washed 1-2 more times under the same conditions until the supernatant was almost colorless. The final treated thylakoid membranes were resuspended in 20 mM Bis-TRIS, pH 6.5, 10 mM MgCl_2 , 20 mM NaCl, 15 mM MgSO_4 , and 1.5% (w/v) Taurine, and then assayed spectrophotometrically for Chl *a* using an $\epsilon_{663\text{nm}} = 86 \text{ mM}^{-1} \text{ cm}^{-1}$ after pigment extraction with 80% (v/v) acetone³⁷. The thylakoid membranes were next diluted to 0.15 mg Chl/ml with the same buffer, treated with 1% (w/v) n-dodecyl β -D-maltoside (DM) for 1 h at 4°C , and centrifuged at $9000 \times g$ for 10 min at 4°C . The resultant supernatant was loaded onto a weak anionic-exchange, DEAE (Toyopearl TSK DEAE 650s) column, pre-equilibrated with 20 mM Bis-TRIS, pH 6.5, 10 mM MgCl_2 , 20 mM NaCl, 15 mM MgSO_4 , 1.5% (w/v) Taurine, and 0.03% (w/v) DM. The bound green material was washed with [about three column volumes of](#) the same buffer and eluted with a 90-550 mM MgSO_4 continuous gradient in the same buffer. Fractions at around 250 mM MgSO_4 (2 mL each) with maxima at

671.5 nm (fractions with higher wavelength peaks contained some PSI core contamination as assayed by antibodies against the PSI core A protein subunit and were discarded) were saved and dialyzed overnight against 20 mM Bis-TRIS, pH 6.5, 10 mM MgCl₂, 20 mM NaCl, 15 mM MgSO₄, 1.5% (w/v) Taurine, and 0.01% (w/v) DM. After concentrating about 10 times with Centriprep tubes (30000 MWCO, Millipore), the sample was loaded onto a continuous sucrose gradient in buffer containing 20 mM Bis-TRIS, pH 6.5, 10 mM MgCl₂, 20 mM NaCl, 15 mM MgSO₄, 1.5% (w/v) Taurine, 0.01% DM, 0.5 M sucrose, and 4 M GuCl. To make the gradient, 10 mL of buffer solution (supplemented with 0.5 M sucrose) was put into each of xxx ultracentrifuge tubes, frozen at -20°C for at least 12 h, and then slowly thawed at 4°C. This procedure formed a sucrose gradient with higher density sucrose at the bottom of the tubes. The concentrated sample from above was supplemented with 4 M GuCl, 0.5 mL of sample was loaded onto each tube, and the tubes were then centrifuged at 180000 × g (SW 41Ti rotor, Beckman) for 6 h at 4°C. Two main green bands were observed in the gradient. The band at higher density (faster mobility) was removed and dialyzed overnight against 20 mM Bis-TRIS, pH 6.5, 10 mM MgCl₂, 20 mM NaCl, 15 mM MgSO₄, and 0.01% (w/v) DM. It had an absorption maximum at 671.5 nm. This preparation was then loaded onto a new DEAE 650s chromatography column, pre-equilibrated with the same dialysis buffer. Under these conditions CP43' passed straight through the column, and it was subsequently loaded onto a Q-Sepharose (a strong anionic-exchange resin; GE Healthcare) column pre-equilibrated with 50 mM TRIS-HCl, pH 7.8, and 0.03% (w/v) DM. The column was washed with the same buffer, and then with buffer supplemented with 50 mM LiClO₄. After washing, green material was eluted with a 50-300 mM LiClO₄ gradient in the same buffer. The fractions obtained at around 200 mM LiClO₄ were pooled and constituted purified CP43' complex. The room temperature absorption

maximum and the 77K origin fluorescence band of pure CP43' ring material were at 671.5 and 685 nm, respectively.

2.3. Isolation of PSI-CP43' supercomplex. The isolation of PSI-CP43' supercomplex was performed as described above with some important modifications. Wild type *Synechocystis* PCC 6803 was used instead of the *psaFJ*-depleted mutant since the former contains much more PSI under iron-starvation conditions. Everything was done as explained before, except that only the fractions from the first DEAE column at around 350 mM MgSO₄ (with absorption peaks at around 673 nm) were collected, concentrated, and loaded onto the sucrose gradient. After centrifugation, two main green bands were observed. The lower one was collected and dialyzed against buffer containing 20 mM Bis-TRIS, pH 6.5, and 0.01% (w/v) DM. This sample constituted the PSI- CP43' supercomplex, which had an absorption peak at 673 nm and two low-temperature fluorescence bands. The smaller fluorescence band was at 686 nm (due to CP43') and the larger one was at 722 nm (due to PSI).

2.4. Spectroscopic measurements. Details about the measurement setup were described elsewhere.^{6,28} Here, only a brief description is given. A Bruker HR125 Fourier transform spectrometer was used to measure the absorption and HB spectra. In absorption and nonresonant HB, the resolution was set at 4 cm⁻¹. For resonant HB, spectral resolution of 0.5 cm⁻¹ was used. The fluorescence spectra were collected by a Princeton Instruments Acton SP-2300 monochromator equipped with a back-illuminated CCD camera (PI Acton Spec-10, 1340 × 400). All emission spectra were obtained with a resolution of 0.1 nm. The laser source for both nonresonant HB and fluorescence was 496.5 nm, produced from a Coherent Innova 200 argon ion laser. In the resonant HB and fluorescence excitation experiments, the tunable wavelengths came from a Coherent CR699 ring dye laser pumped by a Millennia 10s diode-pumped solid state

laser at 532 nm from Spectra-Physics. With laser dye LD 688 (Exciton), the spectral range of 650 to 720 nm was available with a line width of 0.07 cm^{-1} . The power from the ring laser output was stabilized with a Laser Power Controller (Brockton Electro-Optics Corp.). The laser power in the experiments was precisely set by a continuously adjustable neutral density filter. All the experiments were performed at 5 K inside a Janis 8-DT Super Vari-Temp liquid helium cryostat. The sample temperature was read and controlled with a Lakeshore Cryotronic model 330 temperature controller. We emphasize that all nonphotochemical holes disappear after the annealing process, revealing an absorption spectrum identical to that prior to burning and thus proving that no irreversible photochemistry takes place. This is routinely verified by comparing the pre-burn absorption spectrum and the spectrum collected after the sample was annealed to 150 K and cooled back to 5 K.