Characterization of a novel Photosystem I–LHCI supercomplex isolated from Chlamydomonas reinhardtii under anaerobic (State II) conditions

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Abstract A novel supercomplex of Photosystem I (PSI) with light harvesting complex I (LHC I) was isolated from the green alga Chlamydomonas reinhardtii. This novel supercomplex is unique as it is the first stable supercomplex of PSI together with its external antenna. The supercomplex contains 256 chlorophylls per reaction center. The supercomplex was isolated under anaerobic conditions and may represent the State II form of the photosynthetic unit. In contrast to previously reported supercomplexes isolated in State I, which contain only 4 LHC I proteins, this supercomplex contains 10–11 LHC I proteins tightly bound to the PSI core. In contrast to plants, no LHC II is tightly bound to the PSI–LHCI supercomplex in State II. Investigation of the energy transfer from the antenna system to the reaction center core shows that the LHC supercomplexes are tightly coupled to the PSI core, not only structurally but also energetically. The excitation energy transfer kinetics are completely dominated by the fast phase, with a near-complete lack of long-lived fluorescence. This tight coupling is in contrast to all reports of energy transfer in PSI–LHCI supercomplexes (in State I), which have so far been described as weakly coupled supercomplexes with low efficiency for excitation energy transfer. These results indicate that there are large and dynamic changes of the PSI–LHCI supercomplex during the acclimation from aerobic (State I) to anaerobic (State II) conditions in Chlamydomonas.

Keywords: Anaerobic; Chlamydomonas; PQ oxidized; PQ reduced; PSI–LHCI Supercomplex; State I and II; State transitions

1. Introduction

Photosynthetic organisms have developed several mechanisms to adapt to varying light environments. These mechanisms serve to prevent damage under high light conditions, or to maintain high photosynthetic productivity under low light, or under varying spectral quality. One of the most dynamic of such adaptations is referred to as a state (S) transition. During state transitions, the composition of the photosynthetic antenna is remodeled to acclimate to different light conditions. In oxygenic photosynthesis, the Photosystem (PS) I and PSI reaction center complexes act in series to drive non-cyclic electron transfer from water to NADP. The light harvesting antenna of PSII, termed LHCII, contains more chlorophyll (Chl) \(b\) than the light harvesting antenna of PSI, termed LHCI, resulting in a preferential absorption of light at different wavelengths \([1]\). This should be expected to cause an imbalance in the excitation of PSII and PSI, leading to a decrease in the quantum yield of non-cyclic electron transfer. Such a decrease is, however, not observed because of a short-term acclimatory response called a state transition \([1,2]\). Light conditions that preferentially excite PSII lead to a State II transition into State II, which results in a redistribution of excitation energy to PSI \([2]\). The molecular mechanism of state transitions involves the activation of a kinase when the PQ pool becomes reduced \([3]\). The kinase phosphorylates subunits of LHCII, which then become disconnected from PSII. The phosphorylated LHCII is thought to migrate laterally in the membrane to become more closely associated with PSI. This results in an increase in the fraction of total absorbed light energy that is delivered as excitation energy to PSI, thus compensating for overexcitation of PSII under State II conditions. In Chlamydomonas, the switch from State I to State II also results in an increase in cyclic electron flow around PSI \([4]\).

While the structure of PSI from cyanobacteria has been determined by X-ray crystallography at atomic resolution \([5]\), the structural information of plant PSI–LHCI is based on a medium resolution X-ray structure at 4.4 resolution \([6]\) of the PSI–LHCI complex from pea in combination with a recent computational model \([7]\).

The structure showed the core of PSI, which is highly conserved between cyanobacteria and higher plants, is flanked...
2. Materials and methods

2.1. Isolation of PSI–LHCI supercomplexes

*Chlamydomonas* cells were grown following standard procedures. After 48 h, the cells (OD$_{650nm}$ ~ 1) were either shaken in the dark (30 min of anaerobic treatment) or bubbled with N$_2$ for 30 min to oxidize the PQ pool (State I conditions) or were bubbled with N$_2$ for 30 min to reduce the PQ pool (State II conditions). Cells were then broken and thylakoid membranes isolated according to [8] and resuspended in 200 mM sorbitol/5 mM Tris–HCl, pH 8.0/5 mM CaCl$_2$. PSI–LHCI supercomplexes were isolated as previously described [9]. Following density centrifugation, the bands were collected and diluted with 3 volumes of 5 mM Tris–HCl, pH 8.0, 0.05% n-dodecyl-$\beta$-maltoside and 5 mM CaCl$_2$. The diluted samples were then concentrated using a Centricon 100 (Amicon) at 4000 g in a Sorvall SS–34 rotor at 4 °C.

2.2. SDS-denaturing gel electrophoresis and in-gel trypsin digestion

For the analysis of PSI–LHCI protein composition of *Chlamydomonas*, samples were size fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Equal Chl concentration (12 µg) was loaded on each lane. The PSI-LHCI supercomplexes were solubilized in 2% SDS and 0.1 M dithiothreitol, and gel was stained with Coomassie brilliant blue R250. The gel fragment was diced into 1 mm$^3$ fragments and washed three times with 400 µl of 50% acetonitrile/50 mM Tris–HCl, pH 8.0, for 15 min to remove the Coomassie blue dye. Gel fragments were then soaked in a SpeedVac for 20 min at room temperature. Dried gel slices were rehydrated in 40 µl of 50 mM Tris–HCl, pH 8.0, containing 400 ng of porcine trypsin (Princeton separations, sequencing grade). The samples were digested for 18 h at 37 °C, extracted with 50% acetonitrile/5% trifluoroacetic acid for 30 min and dried on a SpeedVac.

2.3. MALDI-TOF mass spectrometry (MS) and database searches

Tryptic peptides were desalted using C18 ZipTips (Millipore) and eluted with 10 µl of 50% acetonitrile/0.1% trifluoroacetic acid. 2 µl aliquots of the sample were mixed with 1.5 µl of matrix (a saturated solution of 3-cyan4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid) and a droplet of the mixture (about 1.5 µl) was spotted on the sample plate and dried. Mass spectra were acquired on an Applied Biosystems Voyager DE-STR mass spectrometer and analyzed with Data Explorer software. From the monoisotopic masses obtained, the amino-acid sequences of matching tryptic fragments of *Chlamydomonas* PSI–LHCI proteins were obtained using the MS-Fit program of the online Protein Prospector software package (UCSF, San Francisco) and the ExPASy Proteomics Server.

2.4. Circular dichroism measurements

Circular dichroism (CD) spectra were measured in a Jasco 710 Spectropolarimeter. The optical path length of the cell was 1 cm, and the distance of the sample from the photomultiplier was 5 cm. The spectra were recorded in 1 nm steps with an integration time of 0.3 s and a band-pass of 2 nm.

2.5. Fluorescence spectroscopy

Low-temperature (77 K) spectra of fluorescence emission excited at 436 nm were measured using a Jobin Yvon-Spxc spectrofluorometer. Low-temperature spectra were measured in the presence of 60% glycerol. The Chl content of the samples was adjusted to 5 µg/mL.

The time-resolved fluorescence of the PSI–LHCI supercomplex was measured using the time-correlated single-photon counting (SPC) technique, essentially as described elsewhere [10,11]. Briefly, samples were excited at 590 nm by ~10 ps laser pulses at a repetition rate of 7.6 MHz. In order to avoid singlet–singlet annihilation, the laser intensity was attenuated to less than 5 × 10$^{10}$ photons cm$^{-2}$ pulse$^{-1}$. Kinetics were measured in the 650–780 nm region with a 10 nm step, deconvolved with an instrument response function (fwhm = 100 ps), and fitted to a sum of exponentials $\sum A_i\tau_i e^{-\tau_i}$, where $A_i$ and $\tau_i$ are the relative amplitude and lifetime of the ith exponential component, respectively. The quality of the fit was judged by weighted residuals and the global $\chi^2$ parameter. Following a global analysis of the fluorescence kinetics, fluorescence decay-associated spectra (FDAS) were constructed by plotting $A_i(\lambda)$ against the emission wavelength $\lambda$ for each $i$, with positive and negative amplitudes in DAS representing a fluorescence decay and a fluorescence rise, respectively. The fluorescence components were scaled to the room-temperature steady-state fluorescence to produce the spectra shown in Fig. 5.

3. Results and discussion

In order to isolate as pure as possible forms of the PSI–LHCI supercomplex in State I or State II form, *Chlamydomonas* cells were adapted to anaerobic conditions that completely reduce the plastoquinone (PQ) pool, or were bubbled with air in the dark to oxidize the PQ pool. It has clearly been demonstrated previously that anaerobic conditions drive the cells into State II [13]. Following sucrose density centrifugation of solubilized thylakoid membranes, three bands were clearly resolved (Fig. 1). The upper band, band A, contains LHC proteins and PSII. Bands B and C contain the LHCI–PSI supercomplex. Under oxidized conditions, which promote State I conditions [4] band B predominated over band C, whereas under reduced conditions, which promote State II [4] band C predominated over band B. In the case of PQ-reduced conditions, there was a significant amount of free orange pigments observed on the top of the tube, indicating that carotenoid synthesis was taking place during the 30 min of anaerobic treatment. In the Stt7 mutant, which does not perform a state transition, [3] band B predominated and essentially there was no band C under both aerobic and anaerobic conditions, as would be expected if the state transition is blocked (not shown). These results clearly show that band B and band C represent different forms of the PSI–LHCI supercomplexes present under State I and State II conditions, respectively. Thus, band B should be the State I (SI) form of the supercomplex, and band C the State II (SII) form of the supercomplex. We will therefore refer to band B as supercomplex-SI, and to band C as the supercomplex-SII throughout the remainder of this text.

To determine the protein content in supercomplex-SI and supercomplex-SII, we performed one-dimensional gel electrophoresis and MS analysis of excised trypsin digested proteins. Compared with supercomplex-SI, the supercomplex-SII shows an increase in the accumulation of LHCI proteins, labeled a–e in Fig. 2, but surprisingly does not contain LHCII. Thus, the increase in density in supercomplex-SII is exclusively due to...
an increase in the complement of LHCI proteins. A number of different LHCI proteins were identified by analysis of mass fragments. The mass fragments were compared to the predicted sequence of known *Chlamydomonas* Lhca proteins [14,15], Table 1. Scanning densitometry of the gels indicate a relative increase in the amount of all Lhca proteins in band C relative to band B, with the exception of Lhca1 (Table 2). Further analysis is under way to determine the precise extent of the changes, and also to determine whether any of the LHCI peptides are post-translationally modified.

The conclusion reached above is consistent with the observed decrease in the Chl a/b ratio from 5.6 in supercomplex-SI to 4 in supercomplex-SII, if we note that the LHCI proteins are enriched in Chl b relative to the PSI core [9]. The supercomplex-SI preparation is heterogeneous and shows strong variations of the antenna size between preparations with an average antenna size of 180 ± 25 Chls/P700. In contrast, supercomplex-SII could be isolated to homogeneity with an antenna size of 256 ± 3 Chls and is stable for two weeks at 4 °C.

If we assume that *Chlamydomonas* supercomplex-SI is similar to the pea PSI–LHCI, for which the structure has been determined at 4.4 Å resolution [6] and which contains 111 Chls in the core and 14 Chls per LHC, we can estimate the number of LHCI peptides associated with our preparations. The supercomplex-SI contains approximately 180 Chls. If we assume that 111 of these are in the core, then 69 are located in the LHCI proteins. If 14 Chls are present per LHCI protein, then 4–5 LHCI proteins are present, making the supercomplex-SI roughly similar in size to the PSI–LHCI supercomplex whose...
structure was solved by Ben-Shem et al. [6]. Likewise, supercomplex-SII contains about 256 Chls, 145 of which would be located in the LHCI proteins. If we again assume 14 Chls per LHCI subunit, then the supercomplex-SII contains 10–11 LHCI proteins.

The ability to isolate two distinct forms of the PSI supercomplex allows us to begin to characterize the supercomplex-SII and to understand how Chl composition and excitation energy transfer may be modified under State II conditions. Emission spectra (77 K) of PSI–LHCI supercomplexes show two dominant peaks at 684 and 708 nm (Fig. 3). The long-wavelength peak at 700 nm is shifted to longer wavelengths in the supercomplex-SII, indicating an increase in red-shifted Chls, probably due to increased amounts of LHCI peptides that bind the long-wavelength Chls.

The visible CD spectroscopy is a sensitive monitor of intramolecular pigment–pigment interactions and/or pigment–protein interactions. In the Qy region, there are two negative peaks at 645, 678 nm and one positive peak at 668 nm (Fig. 4), similar to previous reports [16,17]. The two major bands at 668 and 678 nm are due to Chl dimers caused by the excitonic interaction of Chl $a$ in PSI–LHCI supercomplexes, while the negative peak at 645 nm is the characteristic of Chl $b$ [16]. In the Soret region, the positive band peaking at 443 nm originates from Chl $a$, while the negative peak at 460 nm is characteristic of Chl $b$ (Fig. 4). The negative peak at 470 nm is prominent only in thylakoid preparations, and originates from LHCII [18,19]. Thus, the CD spectra confirm that both supercomplex-SI and supercomplex-SII contain LHCII and lack any major contributions from LHCII.

This does not rule out a role of LHCII reorganization causing an increase in excitation energy transfer to PSI, because the energy transfer process does not necessitate tight binding of LHCII. The differences in the peak intensity are probably

<table>
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<th>Band</th>
<th>Gene</th>
<th>(Total density is in OD)</th>
<th>PQ oxidized</th>
<th>PQ reduced</th>
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<td></td>
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<td>Band A</td>
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<td></td>
<td>LHCII</td>
<td>76.5</td>
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<td>13</td>
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<td>a</td>
<td>Lhca6</td>
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<td>b</td>
<td>Lhca6</td>
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Bands A, B and C are shown in Fig. 1.

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Fig. 3. Low temperature (77 K) fluorescence emission spectra of isolated PSI–LHCI supercomplexes from *Chlamydomonas*. Excitation wavelength was 436 nm and spectra were normalized the emission value at 684 nm. The excitation and emission slit widths were set at 5 and 2.5 nm, respectively.

Fig. 4. Visible CD spectra of isolated PSI–LHCI supercomplexes. The Chl content of the samples was adjusted to 10 μg/mL. CD was measured in absorbance units. However, for easier comparison, the data are plotted in relative units. Experimental conditions are given in Section 2.
the result of differences in pigment content or pigment interactions resulting from the different absorption cross-sections in the PSI–LHCl forms present in supercomplex-SI and SII.

Fluorescence kinetics were measured by single-photon counting from eight different preparations of PSI–LHCI supercomplexes of SI and SII. For both states, decay curves were fit to a sum of four exponentials (Fig. 5). The fastest component (52 ± 19 ps for SI, 53 ± 8 ps for supercomplex-SII) probably represents an overall excitation decay in the antenna due to photochemical trapping. Two intermediate components (250–850 ps and 2.5–3.5 ns) contribute decay amplitudes of less than 10% and are suspected to arise from partially uncoupled pigments or LHCl proteins. The slowest component (5–6.5 ns) probably comes from uncoupled pigments. When the FDAS are compared, the most striking difference between supercomplex-SI and -SII is that the contribution of the slower phases in the SII supercomplex is much smaller and can probably be neglected. We propose that the fastest (~50 ps) component arises from photochemical trapping in intact PSI–LHCI supercomplexes, and that the nanosecond phases are a result of partially uncoupled complexes or detached pigments. The tight coupling of the antenna system to the PSI core in supercomplex-SII as revealed by the 53 ps trapping time, shows that these are intact (in contrast to the heterogeneous supercomplex-SI), suggesting that algae achieve a better structural coupling between the PSI core and the LHC proteins under SII conditions. This is sensible from a functional point of view—the energy supply of PSI may be rate-limiting for photosynthesis under light limitation and other stress conditions, whereas no tight coupling is needed in SI, where the energy supply for PSI is plentiful.

This conclusion is functionally reasonable, since under anaerobic conditions excitation energy should be channeled more efficiently to PSI. When the cells are in oxidizing conditions (State I), energy flow to PSI is not a limiting factor, and there is less need for an efficient, stable extrinsic antenna system for PSI.

The second important feature of the SPC data is the much larger degree of heterogeneity between different sample preparations of PSI from supercomplex-SI in contrast to supercomplex-SII. Although their average trapping times are similar (52.1 ps for supercomplex-SI, 53.0 ps for supercomplex-SII), the distribution of trapping times ranges from 34 to 71 ps for supercomplex-SI, compared to a range of 46–61 ps in supercomplex-SII. In addition, the shapes of the FDAS from independent supercomplex-SI preparations are more heterogeneous than those from independent supercomplex-SII preparations (not shown).

It is interesting to note that, despite the similar average trapping times for both PSI–LHCI forms, the antenna size for the supercomplex-SII sample is about 44% larger, based on the antenna size determination by millisecond flash spectroscopy. Clearly, a larger antenna does not necessarily mean longer average trapping times—the superior structural stability of this new PSI–LHCI supercomplex-SII comes with superior energetic coupling. This notion is supported by results of a theoretical modeling study of the pea PSI–LHCI supercomplexes [20], where the rate of excitation energy transfer depends mainly on the orientation of gap Chls and the distance of the antenna Chls from the RC core, rather than the overall size of the chlorophyll network.

These results demonstrate that the PSI peripheral antenna organization is remarkably modified in Chlamydomonas under State I and II conditions. The novel supercomplex-SII is the predominant form in anaerobically grown cells and can be considered the SII form of PSI–LHCI. This reorganization occurs on a very short timescale and likely accounts for a large portion of the increased absorption cross-section of PSI in SII and the higher efficiency of excitation energy transfer, which will allow the cells to optimize their antenna system for growth and survival. These results indicate that the mechanism of the changes in PSI light absorption during state-transitions needs to be re-evaluated, at least in Chlamydomonas. Whether such structural changes occur in plant PSI remains to be determined. It will also be interesting to see if there are any changes in other PSI proteins.

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References


