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Photosynthetic membrane organization and role of state transition in *cyt*, *cpII*, *stt7* and *npq* mutants of *Chlamydomonas reinhardtii*

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ABSTRACT

In *Chlamydomonas reinhardtii*, cytochrome b_6/f and chlorophyll b binding proteins are important in energy distribution between photosystem (PS)II and PSI. In this study, we have used *C. reinhardtii* mutants deficient in cytochrome b_6/f complex (*cyt*), chlorophyll b binding protein (*cpII*), non-photochemical quenching (*npq*) and LHC II kinase (*stt7*) to study the importance of these proteins in electron transport, phosphorylation, and structural organization of thylakoid supercomplexes under optimum growth conditions. Fast Chl *a* fluorescence studies have shown that lack of CpII and Cyt b_6/f caused reduced photochemical yield (F_v/F_m). The disappearance of I phase in *cyt* mutant showed that electron transfer from Cyt b_6/f to PSI is reduced due to un availability of Q_0 site for docking of PQH2 therefore LHC II kinase was unable to phosphorylate LHCII in *cyt* mutant. Further, blue native gel electrophoresis revealed the differential organization of photosynthetic membrane protein complexes in different mutants. Particularly, LHCII trimerization is more in *cyt* and *stt7* mutants. Also, chemically induced state transition (LHCII phosphorylation) was not observed in *cyt* mutant, however, all other mutants were similar to that of wild type. Based on our results, we propose that the LHCII trimer accumulation and its organization with other complexes are very important in state transitions.

1. Introduction

Photosynthesis is the process in which light energy is converted into chemical energy in terms of ATP and NADPH which can be used up by light independent reactions. The photosynthesis process in both higher plants and green algae triggers when light is absorbed by light harvesting antennae complexes (LHC) of photosystem (PSI) and PSII. Crystallographic studies revealed that the LHCI complex of higher plants contains 4–5 subunits [1]. However, LHCI complex of *Chlamydomonas reinhardtii* is larger and contains 9–14 subunits per reaction centre [2,3]. In which, the LHCII complex consists of type I (Lhcbm3, Lhcbm4, Lhcbm6, Lhcbm8, and Lhcbm9), type II (Lhcbm5), type III (Lhcbm2 and Lhcbm7), and type IV (Lhcbm1) and also comprizes two minor antennas (CP26 and CP29) [4].

Imbalanced light energy causes change in the redox state of intersystem electron transport chain in both PSI and PSII. In order to balance the excitation energy between PSII and PSI, the PQ pool triggers state transition [5–7]. During this process, the peripheral LHCII proteins can shuttle between PSI in the stroma and PSII in the grana in order to optimize overall electron transport [8]. In this process Stt7 (Stn7 is an orthologue present in *Arabidopsis thaliana*) is the key kinase enzyme involved in phosphorylation of LHCII leads state transitions in *C. reinhardtii* [9]. In electron flow chain the kinase is activated in response to redox change in the thylakoid membrane. Also, reduction of the PQ pool, either by unbalanced PSII/PSI activity (in favor of the former) or by the chlororespiratory chain, activates the kinase through a mechanism that requires plastoquinol binding to the Cyt b_6/f complex [10]. The loss or modification of Stt7 or Stn7 blocks the state transitions and LHCII phosphorylation [9,11]. The phosphorylated Pi-LHCII disconnects from PSII and migrates to PSI to balance the energy. This process is reversible when the LHCII kinase is inactivated by oxidation of the plastoquinol where Pi-LHCII gets dephosphorylated by a thylakoid associated phosphatase (TAP38) [12,13], which seems to have constitutive but low activity. Dephosphorylated LHCII moves back to PSII, leading to state I.

PQ pool and Cyt b_6/f plays a crucial role in functional coordination of PSI and PSII during redox mechanism. It is well known that Cyt b_6/f is a membrane bound complex that transfers electrons from lipophilic quinones to hydrophilic plastocyanine. In this process proton are transfered across the thylakoid membrane, consequently develops proton gradient, thereby controlling electron transport. It is now known that Cyt b_6/f also plays a key role in

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state transitions [14]. State transitions acts as a switch between linear and cyclic electron flow in alga [15,16]. In state I, electron transport is linear and the two photosystems work in a series to generate reducing power NADPH and ATP. In some cases, state II, PSII is largely disconnected from the electron transport chain and operates cyclic electron flow around PSI to produce the ATP [16,17]. The major role of state transitions is the balancing/restoration of the intracellular ATP levels required for the carbon fixation by reorganization of the photosynthetic electron transport chain [17–19].

The absorbance cross-sections of the photosynthetic reaction centers are also modulated by long-term responses in algae [20,21]. The role of light-harvesting capacity in balancing the energy and photosynthetic electron transfer to avoid over excitation of the antennae is very well studied in PsaF-deficient strain [22]. Reports indicate that photosystem stoichiometry is usually regulated at PSI, rather than that of PSII [23,24]. Further, the redox state of the PQ pool was shown to regulate transcription rates of the chloroplast-encoded PSI genes, *psaA* and *psaB* during the photoacclimation process of mustard seedlings [25].

In this study, *cpII* (mutation which affected CPII and Chl *b* expression), *stt7* (deficiency of LHCII kinase) and *cyt* mutants (mutation which affected Cyt b_6/f synthesis) have been used to characterize the organization of thylakoids membrane complexes under standard growth conditions and also observed the state transitions. The earlier report of *cpII* mutant of *C. reinhardtii* was devoid of light-harvesting Chl-protein complex (CP) CP II, but had normal Cyt b-559 and displayed all wild type photochemical activities [26]. The Cyt b_6/f complex plays a central role in the photosynthetic electron transfer chain between PSII and PSI. The class of *C. reinhardtii* mutants were deficient in both chloroplast *c*-type Cyt but contained normal amounts of mitochondrial Cyt and a PS II cytochrome [27]. Because the two plastid *c*-type Cyt *f* and *c*6, are encoded in different genomes, may not have affect the expression of the plastid *petA* and nuclear *Cyc6* genes encoding the respective polypeptides. Thus, the normal abundance of mitochondrial cytochromes and PSII function argued against a defect in the cofactor biosynthetic pathway. Further it was reported that a common posttranslational assembly step might be affected in these mutants and also the same group have reported that normal PSII function in Cyt b_6/f deficient mutants [28]. *Npq* mutant which generally does not affect photosynthetic performance at low light conditions and however, in high light conditions the photosynthetic performance is reduced [29]. In case of *stt7* encoded protein plays a major role in phosphorylation of LHCII in low light conditions [9].

Though these mutants have been generated long time back the complete abundance of supercomplexes and their functional relationship in terms of photosynthetic performance has not been studied in optimal growth conditions. Thus, here we studied the organization of photosynthetic components, function and phosphorylation of LHCII in wt and mutants, *cpII*, *npq*, *stt7* and *cyt* in state transitions and optimal growth conditions of *C. reinhardtii*. Here, we reported that the abundance PSI and PSII supercomplexes were varied in different mutants.

2. Materials and methods

2.1. Strain and culture conditions

C. reinhardtii wt (CC-125, mt+ 137c), and mutants *cyt* (CC-3092 F18 mt+), *cpII* (CC-2756 pg-27), *stt7* and *npq* (CC-4101) were grown in controlled optimal growth conditions (light intensity was adjusted to 35 μmol m^{-2} s^{-1} and temperature was maintained at 24 ± 2 °C). Wt. and mutant cells were inoculated to an O.D of 0.4 (1 ml) to 100 ml of growth medium. Cells were grown in Tris–acetate–phosphate (TAP) medium at 25 °C. *wt*, *cpII* and *cyt* mutants were purchased from *Chlamydomonas* center, USA and *stt7* mutant was gifted by Prof. Yuichiro Takahashi, Okayama University, Japan.

2.2. Fast Chl a OJIP fluorescence transient measurements

Chlorophyll fluorescence induction curves were measured using a Plant Efficiency Analyzer (PEA) (Hansatech, Kings Lynn, Norfolk, UK). The fluorescence transients (OJIP) were measured for wt and mutants *cpII*, *cyt*, *npq* upon excitation. Cultures were excited by an array of three light-emitting diodes peaking at 650 nm at a photon flux density of 3000 μmol m^{-2} s^{-1}. Fluorescence was detected by a PIN-photodiode after passing through a long-pass filter (50% transmission at 720 nm).

2.3. State I and state II locked cells preparation

State I locked cells were obtained by treating the cells with 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (sigma) for 10 min under high light 2700 l×. State II locked cells are obtained by treating with 100 mM sodium fluoride, phosphatase inhibitor that prevents the dephosphorylation of pi-LHC II for 50 min and then in 5 μM carbonyl cyanide-*p*-(trifluoromethoxy) phenylhydrazone an uncoupling agent that brings the system to state II by depleting cellular ATP for 10 min. Thylakoids were isolated by the procedure explained in Section 2.4.

2.4. Preparation of thylakoid membranes

Thylakoid isolation from wt and mutants were followed as described by Kargul et al. [30] and Subramanyam et al. [31] with minor modifications. Cells were homogenized by sonication in 25 mM HEPES (pH 7.5, KOH), 0.3 M sucrose, 10 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM NaF and1 mM PMSF. Homogenate was centrifuged at 200*g* for 3 min. The supernatant was centrifuged at 5000*g* for 10 min and the pellet was resuspended in 5 mM HEPES (pH 7.5, KOH), 0.3 M sucrose, 10 mM NaF and 10 mM EDTA. This suspension was centrifuged at 18,000*g* for 10 min and the pellet was resuspended in 20 mM Tricine (pH 7.5), 0.3 M sorbitol, 10 mM $MgCl_2$, 10 mM NaF 5 mM $CaCl_2$ to yield thylakoids.

2.5. Separation of thylakoid membrane proteins by BN/SDS PAGE

First dimension of blue native gel separation was carried out by solubilization of thylakoids in 1% *n*-dodecyl β-d-maltoside (DM) along with the protease inhibitors 1 mM Amino Caproic Acid (ACA),1 mM benzmidine hydrochloride,1 mM PMSF, the gel with 50 mM ACA is run at 0 °C with increasing voltage [32,33]. For second dimension BN gel strips were treated with solubilization buffer; Lamelli buffer: 138 mM Tris–HCl, pH 6.8, 6 M urea, 22.2% (v/v) glycerol, 4.3% (w/v) SDS,5% 2-mercaptoethanol. Second dimension of the BN gel is run in 12.5% SDS gels. The protein spots on second dimension gels are visualized by colloidal coomassie staining method.

2.6. Immunoblotting analysis of thylakoid membranes in order to show the state transitions

Tricine SDS–PAGE was carried out as described by Shägger and von Jagow [34]. The thylakoid proteins were separated on 15% resolving gel and the proteins were transferred on to polyvinyldenedifluoride (PVDF) membrane (Bio Rad) using transblot apparatus (Bio Rad), according to manufacturer's instructions. For phosphorylation studies, blots were probed with rabbit anti-phosphothreonine polyclonal antibodies (1/5000 dilution) purchased from New England Biolabs (CST) to detect proteins phosphorylated at threonine site.

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