Regulation of Reversible Dissociation of LHCII from PSII by Phosphorylation in Plants

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ABSTRACT

LHCII is a crucial light-harvesting pigment/protein complex in photosystem II (PSII) supercomplex. It also participates in the light energy redistribution between photosystems and in the photoprotection via its reversible dissociation with PSII and PSI (photosystem I). This reversible detachment of LHCII is regulated by phosphorylation of its own and PSII core protein. Under low light conditions, LHCII is phosphorylated and dissociated with PSII core protein complex and combined with PSI, which balances the excitation energy between PSII and PSI; Under high light environment, the phosphorylation of PSII core proteins makes LHCII detach from PSII. The dissociated LHCII presents in a free state, which involves in the thermal dissipation of excess excitation energy. During photodamage, dual phosphorylations of both PSII core proteins and LHCII complexes occur. The phosphorylation of D1 is conductive to the disintegration of photodamaged PSII and the cycle of repair. In this circumstance, the phosphorylation of LHCII is induced by reactive oxygen species (ROS) and then the phosphorylated LHCII migrates to PSI, into the repair cycle of damaged PSII. The ferredoxin (Fdr) and thioredoxin (Tdr) system may play a possible central role in the phosphorylation regulation on LHCII dissociation.

KEYWORDS

Light-Harvesting Complex II (LHCII); Phosphorylation; Photosystems; Photoinhibition; Ferredoxin and Thioredoxin System

1. Introduction

Photosynthetic organisms utilize sunlight energy, CO2 and H2O to synthesize carbohydrates. The photosynthetic process consists of light reaction and dark reaction. In plants, light reaction occurs inside chloroplasts, to generate NADPH and ATP by photosynthetic electron transfer chain and photophosphorylation. The resulting NADPH and ATP are used to convert carbon dioxide into carbohydrates in dark reaction. The electron transfer chain locates in the membrane of thylakoid, containing the two types of photosystems (PSII, PSI) and cytochrome b6-f (Cytb6-f) complexes. NADP production depends on the linear electron transfer involving PSII, Cytb6-f and PSI. Extra ATP is synthesized by the cyclic electron transfer only involving PSI and Cytb6-f. There is a difference in light absorption wavelength between PSII and PSI. With these characteristics of photosynthesis, to acquire the high efficiency of light utilization, plants not only need to coordinate the action between light reaction and dark reaction, but also between PSI and PSI.

Light is the sole energy for plant photosynthesis, but it also damages the photosynthetic apparatus [1-4]. Under fluctuating light or strong light conditions, the imbalance allocation of excitation energy occurs between PSII and PSI, or the captured energy in light reaction beyond the need of dark reaction. The resulting excess energy in photosynthetic apparatus damages PSII complexes and reduces the photosynthetic efficiency. This phenomenon is so-called photoinhibition or photodamage. In order to avoid this injury, plants have developed a series of specific
protection mechanism in chloroplasts during the long-
term evolution [5-7]. This mechanism involves the ar-
chitectural shifts in PSII complexes and thylakoid mem-
brane [8]. One of them is the reversible detachment of 
light-harvesting pigment complex with PSII and PSI core 
plex [9-11]. Under the light condition with changing 
wavelength, the reversible detachment of LHClI complex 
kes the excitation energy redistributed between the 
two photosystems [10,12]. In continuous intensive light 
condition, a number of LHCIIIs dissociate from PSII and 
resent in a free state inside chloroplast stroma, prevent-
ing more excitation energy from destroying the reaction 
centers [13,14]. Several reports show that the detachment 
of LHClII also participates in PSI repair mechanism dur-
ing photodamage [15,16]. In higher plants, the reversible 
dissociation of LHClII with the photosystems is regulated 
by phosphorylation [6,17], and the mechanism of phos-
phorylation according to the light intensity.

2. LHClII Phosphorylation in State 
Transition

The reaction centers of PSII and PSI have different light 
absorption characteristics, their absorption peak, respec-
tively, at 680 nm and 700 nm. As green plants are sub-
jected to light environment with fluctuating intensity, 
PSII and PSI are excited in out-balance and LHCIIIs dis-
placed from the over-excitation photosystems to the less-
excitation photosystem, which is so-called state transition 
[12,18]. Under photoinhibition conditions, state transition 
is also considered as a very important photoprotec-
tion mechanism of PSII [19].

The reversible phosphorylation of LHClII complex re-
gulates the reversible displacement of LHClII between 
two photosystems is regulated by the phosphorylation/ 
dephosphorylation of LHClII complex [17], because the 
phosphorylation state of LHClII changes LH ClII affinity 
with the two different photosystems [6]. As PSII com-
plex is overly excited, LHClII is phosphorylated. And 
then the phosphorylated LHClII dissociates from PSI to 
moves towards PSI, which is called state 2. When PSI is 
excessively excited, the phosphorylated LHClII is de-
phosphorylated and detached from PSI and then returns 
sto PSI, which is called state 1 [11,20]. In unicellular 
organisms, the mobile fraction of LHClII during state 
transitions is approximately 80%, whereas in land plants 
the percent is only 15% - 20% [11].

The major function of photosynthetic electron trans-
port chain is to synthesize NADPH and ATP. In some 
circumstances, plants need to strengthen cyclic photo-
synthetic electron transfer to generate more ATP. State 
transitions are found to participate in the regulation of 
ATP synthesis in *Chlamydomonas reinhardtii* by inhi-
biting chloroplast respiration, the phosphorylation level 
of LHClII significantly increased and accompanied by 
cytb6-f complex movement to PSI, which promoted the 
cylic electron transfer and ATP generation. The similar 
phenomenon was observed in higher plants. The inhibi-
tion of ATP synthesis in tobacco plants induced the state 
transition and also accelerated the cyclic electron flow 
[22].

PSII is a dimeric supercomplex containing changeable 
number of outer LHClII complexes [23]. The LHClII 
consists of trimeric LHClII complexes, from 2 to 4 copies 
[24]. The polypeptides are encoded by the genes of lhcb1, 
lhcb2 and lhcb3 [25]. Lhcb1 and Lhcb2 have been well 
documented to reversibly phosphorylated/dephosphory-
lated, and Lhcb3 has not been found to have any phos-
phorylation site. After phosphorylated and dissociated 
from PSI, LHClII trimer is depolymerized and released 
phosphorylated monomers that binds to PSI [10]. The 
connection between LHClII antenna and PSI core com-
plex is through antenna proteins of CP29, CP26 and 
CP24, which are the products of genes of lhcb4, lhcb5 
and lhcb6 [24,26,27]. In Arabidopsis, three isoforms of 
CP29 are found, named as Lhcb4.1, Lhcb4.2 and 
Lhcb4.3 [28]. Nevertheless, the difference in function of 
these protein isoforms has remained elusive.

The recent studies have revealed the protein kinases 
involved in PSI phosphorylation events [29]. In *Arabi-
dopsis*, two orthologue thylakoid-associated serine/ 
threonine kinases, called STN7 and STT7, were identi-
fied by mutant analysis approach and found to be the 
LHClII phosphokinase [6,9]. They are single-spanning 
membrane domain proteins. Their N-terminal regions 
locate in luminal side and C-terminal kinase domains in 
stromal side in chloroplasts. STN7 is essential for reversi-
ble LHClII polypeptide phosphorylation. This phos-
phorylation is required for state transitions in *Arabi-
dopsis* [30-33]. The loss of STN7 in plants blocks the movement 
of LHClII complex from PSII core complex to PSI core 
complex [30,34]. Low light intensity activates STN7, but 
high light intensity deactivates it [31,35]. The redox state 
of mobile electron carriers between photosystems such as 
plastoquinone (PQH2 and PQ) [35-39] and ferredoxin/ 
The lack of PQH2 at the Qo site inside Cytb6-f complex 
results in STN7 deactivation in dark. Under short-wave-
length light, PSII is over-excited, PQH2 binding to the 
Qo site and causing a conformational change in Cytb6-f, 
which transfers the reduction signal from thylakoid lu-
men to the kinase domain in stromal side and activates 
LHClII kinase, resulting in LHClII phosphorylation [15, 
40]. A new model is proposed by Puthiyaveetil (2011) 
[12]. In his model, when PSI is overly excited, PQH2 
released by Qo site reduces a disulfide bond between 
conserved luminal cysteine residues in STN7 and the
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3. Phosphorylation of PSII Core Proteins in Photoprotection

The reversible dissociation of LHCII from PSII also occurs under high light intensity, but the mechanism differs from that in low light condition. The phosphorylation of core proteins in PSII complex occurs during photoprotection. The original and primary site of photodamage is locked in PSII [1]. In order to avoid this injury, plants need decreasing the excitation energy transferred to PSII reaction centers in high light condition. Therefore, LHCII detachment from PSII and PSI is conductive to this energy-decreasing process. But, under such high light condition, the phosphorylation of LHCII is restricted [20]. Several studies have shown that the ferredoxin and thioredoxin system at the side of PSI the down regulated the LHCII phosphorylation. The protein kinase STN7, which catalyzes LHCII phosphorylation, has disulfide bonds on the luminal side [17]. The disulfide bonds may be reduced to sulfhydryl group by ferredoxin-thioredoxin system, resulting in STN7 inactivation [12]. However, a fraction of LHCII are still detached from PSII under strong light condition, and the detached LHCII present in a free state, not binding to PSI [14]. The free LHCII dissipate the excitation energy via fluorescence release and thermal radiation. This process prevents the excitation energy which is not needed by reaction centers, from entering the reaction centers [14,50,51].

The protein phosphorylation of PSII core complex regulates the detachment of LHCII from PSII under strong light, when the phosphorylation of LHCII proteins is restricted. D1, D2, CP43 and PsbH of PSII core proteins were phosphorylated under high light intensity [14,16,17]. PSII was overexcited in the mutants with loss of STN7 kinase under low intensity of light, but the overexcitation was not found under strong light. The mutant analyses on Arabidopsis indicate that the core proteins of PSII is phosphorylated by the protein kinase STN8, rather specific for these proteins [52,53]. This kinase is different from STN7 in structure. It has no disulfide bond in the luminal side so that the activity is not inhibited by ferredoxin-thioredoxin system. Consequently, core proteins of PSII complex are still phosphorylated by high irradiance condition. Zhang and Xu (2003) [14] found the core protein phosphorylation of PSII can induce the changes in the structure of PSII reaction center. These changes may loose the binding of LHCII with PSII reaction center complex, in favor of LHCII dissociation. When green plants are moved to low intensity of light or dark condition, D1 protein is dephosphorylated by phosphatase [54], LHCII returning to PSI again. This phosphatase localizes in stacked thylakoid membrane and catalyzes the dephosphorylation of D1 in functional PSII.
centers, independent of light, which is different from the phosphatase functioning in PSII repair [55]. In a reverse genetic screen, a PSII core protein phosphatase (PBPC) was identified, which counteracts the activity of STN8 kinases and is required for efficient dephosphorylation of PSII core proteins [56,57].

During high light, PSII and PSI need to prevent the accumulation of excess light energy. The detached LHCII must be prevented from phosphorylation again and blocked to bind PSI. The phosphorylated D1 proteins need to remain in phosphorylation state during high light. In this process, the ferredoxin/thioredoxin system may be a central coordinator (as shown in Figure 2). Under high light, PSII through PSI generates more reduced ferredoxin (Fdr) and thioredoxin (Tdr), which activate STN8 and inactivate PSII core protein phosphatase (PBPC) possibly by kinases. STN7 is also inhibited and prevented to phosphorylate LHCII, not associating with PSI. In spite of lacking of disulfide bond, STN8 activity is not inhibited by ferredoxin-thioredoxin system [52,53], but it may be regulated by phosphorylation [58].

4. LHCII Phosphorylation in Photodamage

In high light intensity, LHCII separates from PSII via the core protein phosphorylation of PSII in plants, and is avoided combining with PSI to prevent photoinhibition, even photodamage [3]. However, a number of studies have shown that, when plants are subjected to strong light condition, the phosphorylation of a fraction of LHCII complex remains to occur [16,39]. This phosphorylation may be due to the production of reactive oxygen species (ROS) in PSII reaction center. ROS serves as an oxidant that damages the PSII reaction centers and oxidizes the sulfhydryl group to the disulfide bond of the inactivated LHCII kinase STN7 in thylakoids membrane, restoring the catalytic activity of the enzyme. Breitholtz et al. (2005) [39] found that, under high light intensity, in both Arabidopsis plants with higher excitation capacity of PSII and with defectiveness in non-photochemical reaction activity, the phosphorylation in LHCII occurred. Because the lower ability of non-photochemical quenching and higher excitation capacity of PSII result in more excess energy accumulation in PSII reaction centers, and the generating hydrogen peroxide and singlet oxygen restored the catalytic activity of the LHCII kinase. As a result, the phosphorylated LHCII migrates to the PSI enriched stromal region from the PSII enriched granal region of the thylakoid membrane and transfers the excitation energy to PSI [16]. Then, what function is the binding of LHCII to PSI and whether the binding leads to the damage to PSI?

The phosphorylation of LHCII induced by the high light intensity may involve in repair mechanism of PSII [15]. Of the core proteins of PSII complex, D1 is susceptible to be injured and the turnover rate is fast. Under prolonged high irradiance, ROS generated by PSII reaction center, can inevitably damage the D1 protein [59]. The rate of photodamage increases as irradiance intensity rises. On the other hand, the newly synthesized functional D1 displaced the injured one in PSII core complex and the repair kept pace with the damage. Otherwise, the structure and function of photosynthetic apparatus were severely disrupted. The repair process proposed includes: 1) disassembly of injured PSII-LHCII supercomplex into PSII and LHCII, and PSII core dimer into monomer; 2) movement of the PSI monomer from the stacked membrane region of thylakoid to the stroma region of non-stacked membrane; 3) D1, D2 and CP43 proteins were dephosphorylated; 4) degradation of injured D1 protein; 5) the newly biosynthesized D1 protein cotranslationally inserted PSII complex [60]. The repair cycle of inactivated PSII is associated with dephosphorylation of phosphorylated and photodamaged D1 and is a light-dependent reaction in vivo [55].

The PSII repair rate was decreased while the generation of ATP was blocked in Spirodela oligorrhiza [61,62] because the synthesis of ATP is indispensable for the D1 protein synthesis. The binding of phosphorylated LHCII to PSI accelerates the cyclic electron transfer and enhances the synthesis of ATP [21]. Consequently, the phosphorylation of LHCII is related to the transient repair of damaged PSII complex in photoinhibition. Murata et al. (2007) [62] proposed in his review that unfavorable environmental factors other than high light majorly inhibited the PSI repair cycle. For instance, high salt, low CO2, low temperature and mild heat stress all blocked de novo synthesis of proteins.

**Figure 2.** A diagram of the ferredoxin and thioredoxin regulation on STN8, PBPC and STN7 during high light. Under high light, PSII through PSI generates more reduced ferredoxin (Fdr) and thioredoxin (Tdr), which activate STN8 and inactivate PSII core protein phosphatase (PBPC) possibly by kinases. STN7 is also inhibited and prevented to phosphorylate LHCII.
During the photodamage, PSII core proteins are also phosphorylated like LHCII complex. These dual phosphorylations may more conducive to the repair of the injured PSII. Within chloroplasts, strong light causes the excitation imbalance between PSII and PSI leading to ROS generation and D1 photodamage. Plants prevent and recover from this damage by rearranging the structure of photosynthetic membrane [63]. The phosphorylation of PSII core polypeptides is of importance when the PSII core complex is rapidly photodamaged during high light stress [64]. The movement of impaired PSII complex from PSII-enriched regions to PSI-enriched regions for restore is facilitated by the core protein phosphorylation. A hypothesis supposed that the phosphorylation of injured D1 protein was a signal for the PSII complex movement to PSI-enriched regions from PSII-enriched regions [65,66]. In the PSI-enriched regions, proteases DegP and FtsH decomposed the damaged D1 protein [65, 66]. In light condition, the decomposition of damaged D1 protein is indeed delayed in the mutants of stn8 and stn7/stn8, other than in the plants of wild type and stn7 [30]. The phosphorylation of PSII core protein participates in the change of thylakoid membrane contexture, which facilitated the transport of damaged PSII complex to the PSI-enriched regions for repair. Lack of STN8 impeded the shift of the stacked thylakoid membrane to non-stacked membrane [67]. The stack of photosynthetic membrane blocks the lateral displacement of thylakoid proteins and restraints the conversion of D1 proteins damaged by strong irradiance [67-69]. The stack of thylakoid membrane was not influenced by the lack of STN7 activity [30]. On the contrary, the translocation of movable LHCII fraction from PSII to PSI and state transition1 were impeded [42,34]. The release of PSII subunits was partially regulated by phosphorylation in PSII membranes [70,71]. Upon the phosphorylation of the PSII core proteins, the thylakoid membrane architecture was switched to a state with more fluid, as a result, promoting the cycle of PSII complex repair [72]. In summary, the dual phosphorylations of PSII core proteins and LHCII complexes enhance the dissociation of damaged PSII core protein with LHCII complexes and the migration of the PSII to stromal regions, via the conformational change of thylakoid membrane.

During photodamage occurs, chloroplasts not only need to prevent the accumulation of excess excitation energy in PSII, but also to repair the damaged PSII (as shown in Figure 3). In photodamage, PSII through PSI generates more reduced ferredoxin (Fdr) and thioredoxin (Tdr). Fdr/Tdr activates STN8 and inactivates PSII core protein phosphatase (PBPC) in PSI membrane region possibly by kinases. The sulphhydryl group to the disulfide bond oxidized by ROS, the inactivated LHCII kinase STN7 become active. Active TAP38 may be inactivated via kinase (different from its activation). In addition, the inactive phosphatase in PSI membrane may be activated by Fdr/Tdr system via kinase for D1 dephosphorylation and repair.

**Figure 3.** A diagram of the ferredoxin and thioredoxin regulation on STN8, PBPC and STN7 under photodamage. When photodamage occurs, PSII produces reactive oxygen species (ROS) and through PSI generates more reduced ferredoxin (Fdr) and thioredoxin (Tdr). Fdr/Tdr activates STN8 and inactivates PSII core protein phosphatase (PBPC) in PSI membrane region possibly by kinases. The sulphydryl group to the disulfide bond oxidized by ROS, the inactivated LHCII kinase STN7 become active. Active TAP38 may be inactivated via kinase (different from its activation). In addition, the inactive phosphatase in PSI membrane may be activated by Fdr/Tdr system via kinase for D1 dephosphorylation and repair.

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sary for PSII repair, because damaged PSII repair depends on the dissociation of complexes. D1 phosphorylation is conducive to prevent excitation energy accumulation in PSII. In addition, under photodamage, PSII produces reactive oxygen species (ROS), oxidizing the sulphydryl group to the disulfide bond and activates the inactivated LHCII kinase STN7. LHCII phosphorylation is not only beneficial to the disassembly of PSII complexes, but also enhances cyclic electron transport to synthesize ATP needed to repair damaged PSII complex. Active TAP38 may be inactivated via kinase, which is different from its activation.

**5. Perspectives**

Recent studies have shown that the regulation of reversible dissociation of LHCII is closely related to the site and amount of phosphorylation, but less is known about how plants differentially recognize the sites and control the amount. The regulation of light intensity to this process may be in a more complicated mechanism. In addition, during photodamage, the phosphorylated LHCII binds to PSI reaction center and transfers extra excitation energy to it, a potential damage. Therefore, we need more information about the mechanism of photoprotection of PSI reaction centers in such circumstance. And we know that the low light intensity condition is in favor of the repair of photodamaged PSII. This repair process, in-
volving the synthesis of D1 proteins de novo, needs extra ATP. But the low light intensity also activates phosphatases, resulting in the dephosphorylation of LHCII and the dissociation from PSII. As a result, the cyclic electron transfer and the synthesis of ATP will be decreased. Therefore, plants need a complicated system to ensure the process of D1 repair. Evidence shows that the phosphorylation regulation of reversible dissociation of LHCII with photosystems at least consists of ferredoxin/thioredoxin system and kinase system. Up to date, we have known little about kinases in this regulation network.

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