Hypothesis

A mechanism for regulation of chloroplast LHC II kinase by plastoquinol and thioredoxin

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Abstract

State transitions are acclimatory responses to changes in light quality in photosynthesis. They involve the redistribution of absorbed excitation energy between photosystems I and II. In plants and green algae, this redistribution is produced by reversible phosphorylation of the chloroplast light harvesting complex II (LHC II). The LHC II kinase is activated by reduced plastoquinone (PQ) in photosystem II-specific low light. In high light, when PQ is also reduced, LHC II kinase becomes inactivated by thioredoxin. Based on newly identified amino acid sequence features of LHC II kinase and other considerations, a mechanism is suggested for its redox regulation.

1. Introduction

Photosynthesis is the conversion of light energy into chemical energy by plants, algae and certain bacteria. The molecular machinery of photosynthesis involves photosystems, which are pigment-protein complexes. Each photosystem consists of a peripheral array of light harvesting antenna pigments, embedded into proteins, and an internal core of the reaction center, where a special pair of chlorophylls initiates the light-driven redox chemistry. In a type of photosynthesis known as oxygenic photosynthesis, two distinct photosystems are connected in series via a cytochrome b$_6$f complex (cyt b$_6$f) [1]. This series connection enables the photosystems to split water and extract electrons, liberating oxygen as a byproduct. Electrons extracted from water are then passed on through a chain of electron carriers, and finally to CO$_2$ to make carbohydrates in the Benson–Calvin cycle of carbon fixation. Oxygenic photosynthesis is found in plants, algae and cyanobacteria. In plants and algae photosynthesis occurs in cytoplasmic organelles known as chloroplasts, while in cyanobacteria photosynthesis takes place on internally folded regions of the plasma membrane.

The two photosystems of oxygenic photosynthesis are photosystem II (PS II) and photosystem I (PS I). Due to their differing pigment compositions, PS II and PS I show unique light absorption properties. Absorption of light by PS II is confined mainly to shorter wavelengths than is the case for PS I [2]. Since PS II and PS I are connected in series, for efficient photosynthesis, these two photosystems should convert light energy at an equal rate. In natural environments, the photosynthetically active light is often enriched in particular wavelengths of light, which favors one photosystem over the other. Preferential excitation of individual photosystems causes imbalance in photosynthetic electron transport. Under such light conditions, plants, algae and cyanobacteria employ state transitions, an acclimatory response to changes in light quality. State transitions correct the imbalance in excitation energy distribution by redistributing excitation energy in favor of the rate-limiting photosystem [3,4]. In plants and green algae reversible phosphorylation of the chloroplast light harvesting complex II (LHC II) [5,6] underlies the molecular mechanism of state transitions [7,8]. The protein kinase that phosphorylates LHC II is regulated by the redox state of the interphotosystem electron carrier, plastoquinone (PQ) [9]. In light conditions that preferentially excite PS II, the PQ pool becomes reduced, and the reduced PQ, plastoquinol (PQH$_2$), activates the LHC II kinase [7,8]. The LHC II becomes phosphorylated and the phosphorylated LHC II migrates from PS II towards PS I and transfers excitation energy in favor of PS I [10,11]. This
acclimatory response to PS II-specific light is called state 2. In the complementary light condition, that is when PS I is primarily excited, the PQ pool becomes oxidized. The LHC II kinase is inactive in this light condition and an LHC II phosphatase dephosphorylates phospho-LHC II [12]. The dephosphorylated LHC II then returns to PS II from PS I and redistributes excitation energy to PS II. This adaptive condition to PS I-specific light is state 1. The transitions between these two adaptive states are called state transitions.

Subsequent to the discovery of PQH₂ activating the LHC II kinase [9], it was shown that the electron transfer complex cytochrome b₆f was required for this activation [13–15]. Further research revealed that the quinol oxidation site (Qo site) in cytochrome b₆f controls the activation of the LHC II kinase [16,17]. It thus became clear that quinones acting at one of the quinone-binding sites in cytochrome b₆f, rather than the free, diffusing quinones in the thylakoid membrane, activate the LHC II kinase. A second mode of regulation of LHC II kinase was discovered when the LHC II kinase was found to be in an inactive state in high light [18]. Since the PQ pool exists in a reduced state in high light and the reduced PQ is known to activate the LHC II kinase [9], this inhibition of LHC II kinase was difficult to explain in terms of regulation by PQ. For this regulation, however, the redox state of the stromal electron carrier thioredoxin turned out to be crucial, as the ferredoxin–thioredoxin system [21] or as Stn7 in Chlamydomonas reinhardtii kinase became apparent, genetic analysis of the green alga Chlamydomonas reinhardtii uncovered the identity of the LHC II kinase [21,22]. The LHC II kinase is a serine/threonine kinase known as Stt7 in Chlamydomonas [21] or as Stn7 in Arabidopsis [22]. Stt7/Stn7 has a single transmembrane domain, a lumen-located N-terminal stretch of residues, and a stromally-exposed kinase domain [23].

2. The current model of LHC II kinase regulation

The current model for the activation of LHC II kinase by PQH₂ suggests a mechanism based on the movement of the Rieske iron sulfur protein within the cytochrome b₆f complex [17,24,25]. The Rieske iron sulfur protein is an electron carrier located between PQH₂ and cytochrome f in the photosynthetic electron transport chain. This regulatory model of LHC II kinase, mainly derived from mutational and inhibitor studies of the cytochrome b₆f in Chlamydomonas [17,24], proposes that upon binding of PQH₂ at the Qo site of cytochrome b₆f complex, the Rieske iron sulfur protein moves from a distal position to a proximal position. This movement causes a conformational change in cytochrome b₆f that activates the LHC II kinase. These distal and proximal positions of the Rieske iron sulfur protein refer to the positioning of the surface-exposed head domain with respect to the thylakoid membrane during electron transport. In its distal position, the iron-sulfur cluster of the Rieske protein is distant from the membrane and close to cytochrome f; alternatively, in its proximal position, the Fe-S cluster is close to the thylakoid membrane and the Qo site. For the thioredoxin-mediated inactivation of the LHC II kinase in high light, it has been suggested that the thioredoxin acts at the two conserved cysteine residues on the luminal side of Stt7/Stn7 [21]. According to this model, these two luminal cysteines are interspersed with four amino acids in Stt7/Stn7 represent a thioredoxin target-site, and the reduction of these two luminal cysteines by thioredoxin makes the kinase inactive.

Thioredoxin is found in the chloroplast stroma and therefore how the luminally-positioned cysteines of Stt7/Stn7 could be reduced by it remained problematic in this model. In order to explain this, it has been further proposed that thylakoid proteins such as CcdA and Hcf164 transmit the thioredoxin signals across the thylakoid membrane from the stroma to the luminal cysteine residues of the Stt7/Stn7 [26–28].

This model of LHC II kinase regulation has several limitations. First of all, this model hinges on conformational change rather than the reducing potential of PQH₂ as the ultimate source of the signal. In cases where quinones are known to signal, in fact, it is always their reducing or oxidizing properties that make up the signal [29–35]. In this model it is also not clear how the movement of the Rieske iron sulfur protein and the corresponding conformational change in cytochrome b₆f as part of the activation mechanism are different from similar movements of these components in the protonmotive Q-cycle and in the analogous cytochrome bc₁ complex. Additionally this model posits that thylakoid proteins such as CcdA and Hcf164 are involved in the transduction of thioredoxin signals from the stroma to the luminal cysteines of Stn7; however, these proteins have well-defined functions in the biogenesis and assembly of the cyt b₆f complex [36–38]. It is difficult to see how CcdA and Hcf164 undertake two entirely different functions – biogenesis of c-type cytochromes and regulation of Stn7 – in the thylakoid lumen.

3. A new model for LHC II kinase regulation

Here I propose an alternate model for Stt7/Stn7 regulation. In PS II-specific low light condition the PQH₂, from the Qo site, directly activates the Stt7/Stn7 kinase by the reduction of a disulfide linkage formed by the conserved luminal cysteine residues in the kinase (Fig. 1). Since the quinone-binding Qo pocket of cytochrome b₆f is a rather restricted channel [39], it may be that the quinol cannot directly access the luminal cysteines of Stn7. In that case, the reducing potential of PQH₂ could be transduced to Stn7 via the Rieske iron sulfur protein. This model (Fig. 1) thus proposes that the trigger for the activation of LHC II kinase is the reduction of its luminal disulfide bond by the PQH₂. It is not known whether this disulfide linkage in Stt7/Stn7 is within one monomer or between two monomers of the kinase. As the Stt7 is found as a dimer in Chlamydomonas [27], it is likely that the disulfide bond is intermolecular. Whether or not the disulfide bond is intermolecular, the model suggested here for LHC II kinase activation applies. The formation of the disulfide bond, which is characteristic of the inactive state of the kinase, could be spontaneous in the lumen or catalyzed by thiol-oxidizing luminal proteins such as the cytochrome c₅₅₃ (cyt c₅₅₃) [40].

This activation model of Stn7, however, presents a specific difficulty in terms of redox potential. The standard midpoint redox potentials (Eₜ) of disulfides in proteins vary, with values such as –89 and –270 mV have been reported for catalytic/regulated disulfides [41,42]. While the disulfides that serve structural roles have more negative potentials, which can be as low as –470 mV [43]. The standard midpoint redox potential of PQ, in contrast, is more positive (+80 mV) [44]. Given the fact that the midpoint redox potential of thiol-disulfide couple is more negative than that of PQ–PQH₂ couple, how might PQH₂ be able to reduce the disulfide bond in Stn7? Since the redox potential of chemical species depend directly on the concentration of their reduced and oxidized forms, it may be possible that the redox potential of the PQ pool swings towards a more negative potential, sufficient for the reduction of the disulfide bond, when the ratio of PQH₂ to PQ increases significantly in PS II-specific light condition. Additionally, the principle that a weak reductant (PQH₂ here) will be able to reduce a strong reductant (disulfide in Stn7) if the concentration of the former is higher than that of the latter could hold true here. The extremely low abundance of Stn7 [23] means that the number of...
PQH$_2$ molecules far exceed the number of Stn7 kinases in chloroplast thylakoids. The increase in the reduction potential of PQH$_2$ may also result from the fact that in this light condition the electron acceptors of PQH$_2$ in the high and low potential branches of cyt b$_6$f remain reduced and are unable to accept more electrons from it [16]. The most likely electron acceptor of PQH$_2$ in this condition may then be the Stn7, which is usually found associated with the Rieske iron sulfur protein [23].

If the lumenal cysteines are indeed involved in the activation of the kinase, then, how does the thioredoxin system inactivate Stn7 in high light? A thioredoxin target site, conveniently positioned in the stromally-exposed region of Stn7 would solve this problem. Inspection of the amino acid sequence of Stt7 homologs from representatives of flowering plants, a pteridophyte, a bryophyte and two green algae indeed reveals a hitherto unrecognised, conserved thioredoxin-like CxxxC motif in the stromally-exposed kinase domain of Stn7 (Fig. 2). The reduction of the cysteines of this motif could be responsible for the inactivation of Stn7 by the thioredoxin system. Curiously, this positionally conserved cysteine motif is absent in the Chlamydomonas homolog of Stn7, Stt7 (Fig. 2). However, the amino acid sequence of Stt7 shows two other closely placed cysteines in the middle of the kinase domain (Fig. 2). Since PQ is reduced in high light, the lumenal cysteines of Stt7/Stn7 are also expected to be in a reduced state. The inactivated LHC II kinase in high light will therefore have its both lumenal and stromal thiols reduced. Although this model of Stn7/Stt7 regulation remains to be tested by further experiments, it is simple compared to the current model of LHC II kinase regulation [25,27]. Among other things, this new model does not entail the complicated conformational changes in cyt b$_6$f, which are required by the previous model for the kinase activation, nor does it require the transmembrane transduction of thioredoxin signals to the luminal side of the kinase.

4. Predictions and observations of the new model

(i) The conserved lumenal cysteines of Stt7/Stn7 are crucial for its activation by PQH$_2$. The loss of these cysteine residues will result in the inactivation of Stt7/Stn7. Site-directed mutagenesis studies of these cysteine indeed confirms this prediction [23]. The observation that a low luminal pH activates the LHC II kinase is also consistent with this prediction [45,46], as in highly acidic conditions the cysteine residues of Stt7/Stn7 in the lumen will be protonated and the disulfide linkage broken. Therefore, the effect of acidic conditions on Stt7/Stn7 is similar to the action of PQH$_2$.

(ii) The conserved stromal cysteine motif of Stn7 is critical for its inactivation by the ferredoxin–thioredoxin system in high light. The loss of these residues will abolish the inhibitory effect of thioredoxin on Stn7. Although this prediction remains to be tested, some observations from an earlier study of LHC II kinase regulation [47] seem to bear it out. The greater efficacy of more lipophilic and hydrophobic sulfhydryl-directed reagents in inhibiting LHC II kinase points to regulatory –SH groups that are buried in the kinase [47]. The –SH groups within the stromally-exposed kinase domain of Stn7, as proposed here (Fig. 1), fit neatly with the description of such a buried regulatory site. Furthermore, the demonstration that the inhibitory effect of the alkylating agents on the kinase activity can be relieved by the addition of the adenine nucleotide, ATP, is consistent with the notion that the regulatory –SH groups and the ATP-binding motifs reside in the same region of the kinase [47], and as suggested in Fig. 1. The reduction of cysteines in the CxxxC motif of the kinase domain by thioredoxin in high light may therefore interfere with ATP-binding in Stn7, and consequently inhibiting its kinase activity.
Since the Chlamydomonas Stt7 LHC II kinase does not have the positionally conserved stromal cysteine motif (Fig. 2), it may be that thioredoxin does not inhibit LHC II phosphorylation in Chlamydomonas. So far, the thioredoxin inhibition of LHC II kinase has been demonstrated only in plants [18,48]. A second possibility is that thioredoxin inhibits Chlamydomonas through cysteine residues lying elsewhere in the protein. The role of two closely placed cysteines in the middle of the kinase domain of Stt7 (Fig. 2) in thioredoxin regulation remains to be explored.

If the formation of the disulfide linkage in the luminal side of Stt7/Stn7, which is characteristic of the inactive state of Stt7/Stn7, is catalyzed by cyt c6A and not spontaneous, this then suggests a specific function for the cyt c6A molecule in chloroplasts. The functional role of cyt c6A in photosynthesis has thus far remained elusive; apart from a suggestion that cyt c6A is a general thiol-oxidant in the lumen [40].

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