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Current Understanding of the Mechanism of qE, a Major Component of Non-photochemical Quenching in Green Plants

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Plants dissipate excess excitation energy from their photosynthetic apparatus by a process called non-photochemical quenching (NPQ). The major part of NPQ is energy dependent quenching (qE) which is dependent on the thylakoid pH and regulated by xanthophyll cycle carotenoids associated with photosystem (PS) II of higher plants. The acidification of the lumen leads to protonation and thus conformational change of light harvesting complex (LHC) proteins as well as PsbS protein of PSII, which results in the induction of qE. Although physiological importance of qE has been well established, the mechanistic understanding is rather insufficient. However, recent finding of crystal structure of LHCII trimer and identification of qE mutants in higher plants and algae enrich and sharpen our understanding of this process. This review summarizes our current knowledge on the qE mechanism. The nature of quenching sites and components involved in this process, and their contribution and interaction for the generation of qE appeared in the proposed models for the qE mechanism are discussed.

key words: qE, NPQ, ΔpH, zeaxanthin, PsbS, LHC

INTRODUCTION

Light is essential for the survival of plants, but it is harmful to plants when it is excessive. Light absorption results in the excitation of ground-state chlorophyll (Chl) to its singlet excited state ($^1Chl^*$), which can return to the ground state via one of several pathways (Fig. 1). Excitation energy of $^1Chl^*$ can be transferred to reaction centers for photosynthetic reaction. The rest of energy not used by photosynthesis can be re-emitted as fluorescence or de-excited by thermal dissipation processes. Because the fluorescence yield is reciprocal to both photochemistry and thermal dissipation process, the former process is called photochemical quenching (qE) and the latter is known as non-photochemical quenching (NPQ). However, $^1Chl^*$ also may decay via triplet state ($^3Chl^*$) and convert harmless triplet oxygen to singlet oxygen ($^1O_2^*$), that causes photo-oxidative damage to plants [1]. When we consider the light energy excessive to those used for qP, NPQ can be thought as an important protective mechanism competing with this harmful process.

NPQ is subdivided into three components according to their relaxation kinetics in darkness following a period of illumination, as well as their responses to different inhibitors. The major component, qE, relaxes fast within seconds to minutes and triggered by an increase in the ΔpH. Second component, qT, relaxes rather slowly and is due to the phenomenon of state transition. Third component, qI, is the slowest component and is related to irreversible damages such as the inactivation of photosystem (PS) II reaction center D1 protein [2].

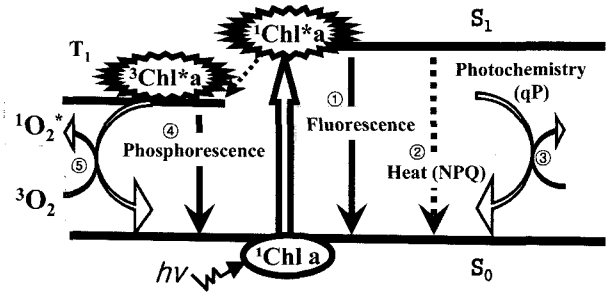

Figure 1. Pathways of $^1Chl^*$ de-activation. When a plant leaf is exposed to the high light, excited $^1Chl^*a$ (S_1) is formed from its ground state (S_0). From there it has several ways to return to the ground state. It can be relaxed by ① fluorescence, by ② energy dissipation processes (NPQ), or by ③ photochemical processes (qP). $^1Chl^*$ can be transformed into $^3Chl^*$and back to its ground state by ④ phosphorescence. ⑤ Due to its long lifetime, $^3Chl^*$ can produce $^1O_2^*$, from harmless triplet oxygen, that leads to photooxidative damage. The yield of $^3Chl^*$and fluorescence depends on the yield of qP and NPQ. Therefore, qP and NPQ help to minimize the production of $^1O_2^*$.

To develop plants tolerant to specific stress conditions, it is very essential to understand how plants efficiently balance photosynthesis and NPQ under various environmental conditions. Although qE is the major component of NPQ that can quench

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up to 80% of the $^1Chl^*$ in green plants and algae [3, 4, 5], the mechanism of qE is still unclear. In this review, we focus on the current understanding of the qE mechanism and try to describe what are commonly agreed among scientists and what are still under debate.

PARAMETERS CONTROLLING qE AND CHARACTERISTICS OF THE qE SIGNAL

The three major parameters controlling qE are the development of transthylakoid proton gradient (ΔpH), the amount of pigments involved in xanthophyll cycle, and the existence of a thylakoid protein called PsbS. These parameters control qE in an integrated way, although the qE signal is mostly disappeared when one of these parameters does not exist.

The qE signal is characterized by (1) the light-induced absorbance changes at 535 nm [6], (2) the shortening of a specific Chl fluorescence lifetime component from ~2.0 to~0.4 ns [7] and (3) the carotenoid cation radical formation [8]. Changes in absorbance and in Chl fluorescence lifetime often reveal the structural changes in pigment–protein complexes of thylakoid membranes.

Requirement for ΔpH

During linear and/or cyclic photosynthetic electron flow, ΔpH is generated, which is an immediate signal for the feedback regulation of the light harvesting, qE. Therefore, the induction or relaxation of qE can be noticed within a few minutes. The requirement for low pH is evidenced by the inhibition of qE by ΔpH uncouplers such as nigericin [9].

An *Arabidopsis* mutant, *pgr1* (proton gradient regulation), with a point mutation in petC encoding a subunit of the cytochrome b_6f complex, entirely lacked NPQ [10]. However, the lumenal acidification generated in this mutant in the low light was above pH 6.0 and was not enough to trigger NPQ, but it was large enough to produce ATP, hence the mutant showed growth rate similar to wild type. Another NPQ deficient mutant called *pgr5* was reported in *Arabidopsis*, which lacked a novel thylakoid membrane protein involved in the transfer of electrons from ferredoxin to plastoquinone [11]. These results revealed that qE is dependent on the lumenal acidification generated by linear and cyclic electron transport and even further the ΔpH requirement is more strict (pH <6).

Requirement for xanthophyll cycle pigments

All organisms in which qE is detectable have a xanthophylls cycle [9]. When leaves are illuminated and the lumenal pH in the thylakoids decreases, violaxanthin deepoxidase (VDE) is activated to convert violaxanthin (Vio) with two epoxide groups to zeaxanthin (Zea) without epoxide group via antheraxanthin (Anthera) with one epoxide group [12].

The level of Zea synthesized is generally known to be highly correlated with the level of qE, but the extent of inhibition of qE by the inhibition of Zea synthesis depends on plant species [13]. Isolated thylakoids devoid of Zea is also reported to exhibit high levels of qE at lower pH values than those generally occurs in vivo [14]. Mutants of *Arabidopsis* and *Chlamydomonas* with defects in VDE gene [15] and tobacco plants with antisense VDE gene [16] did not produce Zea, but they could develop low level of qE. Anthera, the first intermediate produced in Vio deepoxidation, is also known to be involved in qE [9]. In addition to Zea and Anthera, a third xanthophyll molecule lutein also contributes to qE in *Chlamydomonas lor1* mutants [17] and in *Cuscuta reflexa* [18]. Recently, deepoxidation of Vio to Zea has been observed in LHCI, but the Zea formed in LHCI did not contribute to NPQ [19].

In summary, the requirement of xanthophylls is generally true for maximal qE. However, removing all xanthophyll pigments may not abolish qE totally, probably due to the existence of other quenching pigments including Chl and some additional function of ΔpH.

Requirement for PsbS protein of PSII

One of the obligatory requirements for qE is PsbS protein of PSII with molecular mass of 22 kDa [20]. The intensive study of the PsbS protein and its role in qE was started after screening of *AtPsbS* mutant or *npq4-1* of *Arabidopsis thaliana* [5, 21]. These mutants lacked qE as characterized by the light-induced absorbance changes at 535 nm and the shortening of the Chl fluorescence lifetime component. Recently, *OsPsbS* mutant is reported in a model monocotyledon plant, rice [22].

Although it is evident that PsbS protein is necessary for qE, but both *Arabidopsis* [21] and rice mutants [22] completely lacking the PsbS protein, showed normal photochemistry without any visible phenotypes. However, PsbS protein may confer tolerance to variation in light intensity, because the *Arabidopsis* mutant grown under fluctuating light showed less fitness than wild type plants [23]. The exact role and function of the PsbS protein is still unclear, and therefore the mechanism of qE is questionable, too.

QUENCHING SITES FOR qE

To understand the mechanism of qE, we should know where the actual deactivation of the excited Chl takes place. The deactivation of the excited Chl molecule requires quenchers or pigments for its quenching or changes in the environment surrounding the excited Chls. Let's assume that the quenching site of the Chl fluorescence binds pigments, either Chls and/or Car. The candidate pigment-binding complexes include major and minor LHCs. In this section, we will describe their pigment-binding abilities and their possibilities to be quenching sites for qE. We also discuss the possibility of PsbS as a candidate for the quenching site in addition to its questionable pigment binding ability. The structural changes in the pigment-binding proteins will be discussed in the following sections.

Major LHCs

The LHC superfamily includes several pigment-binding complexes with similar polypeptide sequences, structure and function [24]. In higher plants, mainly 10 different pigment-binding antenna proteins are associated with the two photosystems (Lhcb1 to Lhcb6 in PSII and Lhca1 to Lhca4 in PSI). Among them, major LHCs include Lhcb1, Lhcb2 and Lhcb3 that form homo- and heterotrimers [25]. All these three polypeptides are highly conserved among different plant species, suggesting that they have a distinct functional role. From point of view of the role of LHCII for absorption, transfer of solar energy and photoprotection of PSII reaction center, the structure of LHCII has been studied extensively.

Recently, the molecular structure of major LHCs has been determined by X-ray crystallography of stacked two-dimensional crystals with high resolution in spinach [26] and in pea [27]. Each LHCII trimer binds 24 Chl a and 18 Chl b, 12 carotenoids and six lipids. The position of each Chls in a trimer was normalized for trapping, transfer and controlled-annihilation of excitation energy. As quenching pigments more attention is paid to carotenoids. In each LHCII monomer, four carotenoid binding sites (L1, L2, N1 and V1) are present. Lutein binds to L1, L2 sites, which are located at the center of the LHCII monomer, V1 site for Vio has peripheral position, and N1 site for Neo protrudes into the lipid bilayer and V1 is at the monomer interface. According to Morosinotto et al. [28] L1 site is conserved in all antenna proteins, and binding to L2 site is mostly variable and this site can be occupied by Vio or Neo. Molecular environment of Vio binding pocket in LHCII is hydrophobic [27], suggesting that Vio can easily be removed from LHCII.

Based on the pigment-binding ability, especially to the xanthophyll cycle pigments like Vio and the molecular environment of the pigments, LHCII is considered as one of the candidates for the quenching site(s) of qE. However, Andersson and coworkers [29, 30] questioned about the role of LHCII proteins in qE formation, because repression of individual LHCII genes did not induce any phenotypic qE changes.

Minor LHCs

Minor LHCs are also members of LHC superfamily and includes CP29 (Lhcb4), CP26 (Lhcb5) and CP24 (Lhcb6), which are between 210 and 257 amino acid-long and bind five to six Chl *a* and two to five Chl *b* molecules [26, 31], and their sequences are between 29.2 and 48.7% homologous with major LHCs. They are present in the supramolecular antenna of PSII as monomeric forms and located between PSII core complex and the major LHCII [31].

The carotenoid-binding properties of L1 and L2 sites in minor LHCs are similar to those in major LHCs [28], except for CP24, whose L2 site binds only Vio [32]. No minor LHCs is reported to have any carotenoids at V1 binding site [28], N1 site of CP26 bind only with Neo [33], but CP24 did not show any carotenoids in its N1 site. However, N1 site of CP29 can bind Vio as well as Neo.

In the in vitro experiment of [34], xanthophyll exchange capacity occurs at L2 site. In L2 site, minor LHCs contain more bound Vio compared to main LHCII, and among minor LHCs more Vio contents were observed in CP26 and CP24 than in CP29 [34, 35]. In CP26, Vio can bind only in L2 site, and the xanthophyll exchange was the most efficient among the minor LHCs [34]. Therefore, CP 26 may act as the potential quenching site.

Electron microscopy and image analysis shows that LHCII trimers bind to PSII core complex at three sites [32] Depending on the degree of binding to PS II core, the sites are categorized into S (strong), M (moderate) and L (loose). The variation in binding sites of LHCII has been attributed to CP29, CP26 and CP24. Strongly bound LHCII is associated with CP29 and CP26, moderately bound LHCII contained CP29 and CP24 and loosely bound LHCII does not require any CPs for binding to PSII core complex. On the basis of this binding affinity of minor CPs to LHC II and, it can be suggested that CP29 may be a candidate quenching site among minor LHCs. Although the exchange capacity of CP29 was less than that of CP26 in L2 site, N1 site of CP29 can bind Vio (N1 site of CP26 can bind Neo only).

PsbS protein of PSII

To quench Chl fluorescence, (1) PsbS may directly participate in quenching processes if it is able to bind the qE quenchers such as Chls and/or carotenoids, or (2) PsbS may interact with neighboring protein(s) as an allosteric regulator leading to conformational changes in PSII which will induce the production of quenchers [36].

For pigment-binding of PsbS, there is no definitive evidence for the binding of PsbS with Chl. Although PsbS is a member of the LHC superfamily [37, 38], only PsbS apoprotein is stable in the absence of Chl [39]. Amino acid sequence analysis also shows that PsbS protein lacks all of the conserved histidine residues [40] that act as Chl binding ligands in all LHC proteins. However, the PsbS protein isolated from spinach revealed that PsbS binds Chls as well as at least one carotenoid [39], but more recent study by Dominici et al. [41] demonstrated that native PsbS protein as well as recombinant protein do not show any detectable ability to bind pigments. Nevertheless, in the same experimental conditions, LHC proteins maintain full pigment-binding.

Recently, Aspinall-O'Dea and co-authors [42] had success in reconstitution of Zea to PsbS protein in vitro which resulted in a strong red shift in the absorption spectrum and the appearance of the characteristic peaks in the resonance Raman spectrum. In the absence of qE, the appearance of red-shifted Zea absorbing at 523-525 nm, compared with 505 nm, gives rise to a band at 535 nm in the qE difference spectrum. This may be a good evidence to support the hypothesis that this "activated" Zea is bound to PsbS, because reconstituted Zea-PsbS complex shows a similarly strong red shift to 523 nm. This

shift was found to be sufficient to give rise to a 535 nm band in a difference spectrum calculated by subtracting an absorption spectrum of "nonactivated" Zea [43]. The data also provide an explanation for the absence of 535 nm change in the *npq4-1* mutant of *Arabidopsis*, which lacks this protein [5].

PsbS overexpression in tobacco plants showed increased deepoxidation state and NPQ under low light [44]. This result suggest that (1) Zea in lipid phase can inhibit VDE activity, (2) thylakoid lipid phase has limited capacity for xanthophylls cycle pigments and (3) PsbS reduces Zea load to the lipid phase through direct or indirect binding of Zea, reducing feedback inhibition of VDE. So PsbS overexpression plants have higher deepoxidation state and increased NPQ. However, lower deepoxidation state was not observed in *PsbS* knockout rice and *Arabidopsis* mutants (unpublished data).

Location of the PsbS also may be an evidence for this protein as potential quenching site. Dominici et al. [41] investigated the accumulation of this protein in a series of barley mutants affected in PSI, PSII, or LHC proteins and showed that PsbS protein was present in all genotypes analyzed, thus suggesting that PsbS protein is located in the periphery of PSII.

QUENCHERS

Absorption of light by Chls results in the formation of excited singlet state of Chl molecule. Their fluorescence can be efficiently quenched through an energy transfer and electron transfer processes, and for this purpose Chls may involve as well as Cars. The Chl dimers or eximers are also well known powerful quenchers [45].

Quenching of the energy of the excited Chl may proceed by electron exchange mechanism which requires partial orbital overlap, and/or charge-transfer mechanism which requires coplanar π systems [46]. Triplet state of Chls and Cars can quench the excited Chl fluorescence, and the ability of Cars in quenching Chl triplets is also well known phenomena [47, 48]. Therefore, we will describe the quenching abilities of Chl a, Chl b and Cars specially Zea in the following section.

Chlorophylls and their dimers

In a Chl b-less mutant of barley, NPQ is less as compared to wild type plants [49], which indicates that Chl b plays a role in the quenching of Chl fluorescence. The Chls in LHCII may deactivate $^1Chl^*$ by singlet-singlet or singlet-triplet annihilation [46, 47, 50]

Recently, Pascal et al. [51] observed red-shift in fluorescence emission of LHCII crystals and suggested that these quenching centers could be Chl dimer or eximers. The authors also observed a putative quenching center in LHCII crystals at high resolution (2.72 Å) with Chl a molecules on the stromal side of the LHC. This center contains the Chl a pair (Chl a 611/Chl a 612 - according to nomenclature in [51]). Other candidates for quenching centers include a Chl b dimer (Chl b 606 and Chl b 607) and a Chl a and Chl b dimer (Chl a 614 and Chl b 605).

Zeaxanthin

Carotenoid molecules, especially xanthophylls, have a greater role in the dissipation of excess energy. Zea is identified as the main quencher of excess energy. There are two hypotheses for explaining the role of Zea in qE: direct and indirect quenching. The indirect quenching model has been argued by Ruban et al. [6] who claimed that the conversion of Vio to Zea controls over the aggregation state of the antenna complexes that favor quenching. In contrast, the direct quenching model depicts Zea as an acceptor of excitation energy from Chl [52]. The direct quenching mechanism invokes two ways: a) energy transfer to S_1 state of the Zea molecule; b) electron transfer after the formation of a Chl-Car dimer.

The former way was suggested on the belief that the S_1 energies of Zea is lower than Chl Q_y, while the S_1 energies of Vio is not. However at present the experimental evidences suggest that the relative S_1 energies of Zea and Vio is same [53], and both the molecules have S_1 states at higher energy than Chl Q_y [54].

The latter way describes an electron transfer mechanism, via Zea-Chl dimer [46]. However, both of them require presence of Chl and Zea molecules. They theoretically showed that charge transfer between Zea and Chl molecules was possible. Consequently, the charge transfer state corresponds to an excited state, in which an electron is transferred from Zea to Chl resulting in a Zea cation radical and a chlorophyll anion radical. Further, Holt et al. [36] showed by transient absorption measurements of thylakoids in quenched state that when the qE was formed, carotenoid cation radical formation was occurred. The mutant plants of *Arabidopsis*, *npq4-1* and *npq1-2*, both lacking qE, do not show carotenoid cation radical formation; this let authors to suggest that upon qE, the key molecular component involved in energy dissipation in PSII is Zea cation radical.

Other carotenoids

Lutein and beta-carotene are abundant carotenoids in plants. Lutein as well as Zea is necessary to change LHCs from light favoring form to one that dissipates excess energy [55]. It has also been shown that lutein is not essential when Zea is present. Lutein can be replaced by other cartenoids and luten-deficient mutant does not show distinct phenotype [56]. Vio and neoxanthin seems not to function in photoprotection, considering that *Chlamydomonas* and *Arabidopsis* mutant that contain only Vio and neoxanthin are sensitive to light stress [57]. Although the roles of these carotenoids in photoprotection are well known, but the participation of these carotenoids in qE formation is still questionable.

CONFORMATIONAL CHANGES WHICH AFFECT qE

qE involves specific changes in the configuration of Chls and carotenoids as detected by absorption, fluorescence, circular dichroism (CD) and resonance Raman spectroscopies [2]. The separation and configuration of these pigments need to be finely controlled by the structure of pigment-binding proteins. This is achieved by the modification of protein conformation. Change in conformation is associated with light scattering and changes in ultrastructure of thylakoids [58]. As quenching is a bimolecular reaction between two fluorescing molecules or domains [59-61], small changes in the conformation of the proteins [62] cause pigments in specific domains of protein structure to become reversibly configured. In that way, conformational changes lead to specific interactions between pigment molecules, like Chl dimer/eximer or Chl-Car, and then cause the formation of charge transfer states of the interacting molecules.

The conformational changes induced by the protonation of LHCs are also known to induce aggregation of LHCs. In this section, we describe ΔpH-dependent protonation and aggregation of the putative quenching sites in LHCs and/or in PsbS protein of PSII.

Protonation

Why the protonation of the putative quenching sites is important? There are several ways by which a quenching may occur. First, protonation may change conformation of the LHC polypeptides that result in shortening the distance between Chl molecules or between Chl and Zea [63]. Second, protonation may change the local electric field around pigment molecules which promote energy transfer [64]. Third, if the glutamate residues that bind Chl in LHC polypeptides are protonated, they may directly form a quencher with nearby Chl or Car molecules [65], and the same thing may be true for PsbS.

ΔpH-induced-protonation of minor LHCs, CP26 and CP29, has been reported [66]. Most of the evidences for protein protonation were obtained with experiments using DCCD, a protein-modifying agent as well as a powerful and specific inhibitor of qE [67]. DCCD has been shown to react with acidic amino acid residues, in a hydrophobic environment, and is involved in proton translocation. When CP29 subunit of PSII of *Arabidopsis* was mutated at glutamate 166 (E166), it could not bind DCCD, suggesting that protonation of the E166 in normal CP29 lead to a conformational changes triggering qE [68]. Similar quenching may take place in CP26 [66], but not in CP24 [69].

The hypothesis that protonation of the PsbS protein is essential for qE induction is supported by the finding that the qE inhibitor DCCD binds to PsbS protein [41]. Although PsbS purified from spinach or *Arabidopsis* and PsbS expressed in *Escherichia coli* binds DCCD at pH 7.5, PsbS in thylakoids isolated from wild-type *Arabidopsis* plants binds DCCD at pH 5 [70]; but this binding of DCCD was not detected at pH 7.8, showing that DCCD binding is pH-dependent. Conversely, binding of Zea to PsbS protein in vitro was pH-independent [42]. On the basis of these evidences, it has been suggested that in vivo Zea binding to PsbS protein may be regulated by protonation of carboxyl amino acids that induces conformational changes in PsbS [36].

Two pairs of symmetric, conversed glutamate (E) residues (E131 and E235; E122 and E226) on the lumenal side of PsbS protein were important for the functioning of the PsbS [71]. These residues were not involved in pigment-binding, not like the residues in LHC proteins [72]. Single mutations of the residues E122 and E226 led to 60-70% decreases in qE, and the double mutation of these residues caused inhibition of qE, which is the typical phenotype of the PsbS lacking mutant (*npq4-1* mutant of *Arabidopsis*) [71]. These glutamate residues are located in the central region of the two conserved loops in PsbS, and they are the sites for potential proton binding. These idea are supported by the finding that DCCD binding occurred at pH values similar to those found in the lumen during qE induction [71].

Aggregation

There are evidences to support the view that protonation of LHCs leads to dramatic changes in their subunit-subunit interactions, resulting in aggregation of such complexes in vitro. The spectroscopic changes accompanying in vitro aggregation have similar features that were observed during qE induction in thylakoids and intact leaves [2]. The spectroscopic changes also indicates that there are significant alteration in Chl and Car interactions.

Horton and co-workers [73] suggested that quenching does not require aggregation of the LHCII, but quenching was always more when LHCII aggregates formed. It is known that xanthophyll cycle pigments may promote conformational change in both major and minor LHCs. The change in conformation leads to aggregation of LHCs and alter Chl-Chl or Chl-Car interaction and thus an increase in quenching [74, 75]. When an aggregation of LHCII trimers was induced in isolated LHCII complexes, a 1~2 nm red-shift was observed in the LHCII peak at 695 nm and a shoulder at 700 nm was appeared in 77 K fluorescence spectra [76, 77]. These spectroscopic changes may be ascribed to alteration of Chl-Chl interactions.

Crofts and Yerkes [65] reported that Mg^{2+} induced aggregation does not alter energy-dependent quenching. Probably, conformational changes due to Vio or Zea binding are different from the changes in LHCs induced by Mg^{2+} binding. LHCII-LHCII contacts expected in aggregates in vitro may not exist in vivo. Instead, contacts with hydrophobic surfaces like PsbS or lipid may induce the changes equivalent to the in vitro aggregation [73]. LHCII in vivo may exist in a state between two extreme

states, one fully unquenched (non-aggregated) state and a fully quenched (aggregated) state. In vivo supramolecular organization of the PSII antenna as shown by high resolution EM [78] and CD spectroscopy [79] reveals that there are numerous interactions between proteins and the changes in these interactions may be responsible for quenching.

QUENCHING MODELS PROPOSED

The PsbS model

K. K. Niyogi and collaborators [8, 20, 36] suggest that PsbS is the main site of qE. According to this model, the protonation of two carboxyl amino acids of PsbS at low lumen pH is necessary for qE (Figure 2). At low pH, VDE also seemed to be protonated to become an active form to generate Zea. Protonated PsbS activates binding sites for two Zea, resulting in the qE state in which excited energy of singlet Chl is transferred to Chl-Zea heterodimer to form Zea cation radical for non-radiative deactivation of singlet Chl. Excitation energy from LHCII may be transferred to the quenching site in PsbS as described in this model, or it may be deactivated by the LHCII quenching mechanism proposed by Horton and collaborators (described in the following section). Although this model is attractive, pigment-binding ability of the PsbS still questionable.

The LHCII model

Recent view of the LHCII aggregation as a cause for qE induction has been explained by Horton et al. [73]. According to this model, qE occurs in LHCII. Four different states with different conformation exist for LHCII. The conformational changes resembling in vitro LHCII aggregation depend on both protonation of LHC proteins and de-epoxidation of Vio. Binding of carotenoids allosterically regulate qE on the periphery of LHCII. Quenching occurs in LHCII by specific interactions of pigments. In this model, there are spaces for the direct participation of xanthophyll pigments in NPQ and PsbS is preferred as a regulator of qE, rather than a direct quencher.

The hypothesis of "allosteric regulation of NPQ" [73] predicts that only small changes in ΔpH is enough to induce qE, allowing optimisation of electron transport and ATP synthesis, and metabolic control of light harvesting. However, in *Arabidopsis* mutant *pgr1*, it has been shown that for qE formation small trans-thylakoid pH is not enough [10].

On the contrary to this model, Kühlbrandt and coworkers suggested that quenching did not require any conformational changes in LHCII based on crystal structure of the LHCII from pea plants [27]. They suggested a mechanism for regulating xanthophyll cycle-dependent component of NPQ (likely qE). Due to the hydrophobicity of Vio binding pocket [27] and low binding affinity of the Vio [33] to LHCII, they can be easily converted into Zea. Lower pH in lumen activates VDE which converts easily equilibrated Vio (with a pool of free carotenoid in the membrane) to Zea via Anthera and then Zea binds to Vio site in LHCII, which is positioned at the monomer interface and converts a LHCII trimer to an energy sink. Unlike the other two models, the model of Kühlbrandt and coworkers does not require neither conformational changes nor PsbS protein, because authors did not observe any conformational changes in two different LHCII crystals (both in quenched state), although one LHCII crystal from pea was grown at pH 5.5 [27], and the other crystal from spinach was grown at pH 7.5 [26].

Concluding remarks

On the basis of current knowledge, we proposed a modified from [36] model for energy-dependent quenching in plants (Figure 2). Changes in linear and/or cyclic electron flow result in decrease in lumen pH and thus trigger protonation of the VDE and of PsbS protein of PSII. Protonated PsbS protein binds Zea and the protonation leads to aggregation of the LHC and thus conformational changes in thylakoid membrane. These changes cause subtle changes in the distance/orientations between pigments (Chl a, Chl b and Car) and leads to the formation of quenching site(s) which deactivate(s) excited singlet state of the Chl.

More recently, new type of the qE-deficient mutant of *Arabidopsis*, *que1* has been isolated [80]. This mutant shows pH-dependent conformational changes at 535 nm, but not as a result of lumen acidification and xanthophyll cycle. In addition, *que1* mutant does not show reduced level of the PsbS gene expression and mutation of the PsbS, but reduction of the Zea accumulation and electron transport rate was observed instead. Taken together, these findings show that qE is a complex, feedback de-excitation process which can be altered by many factors.

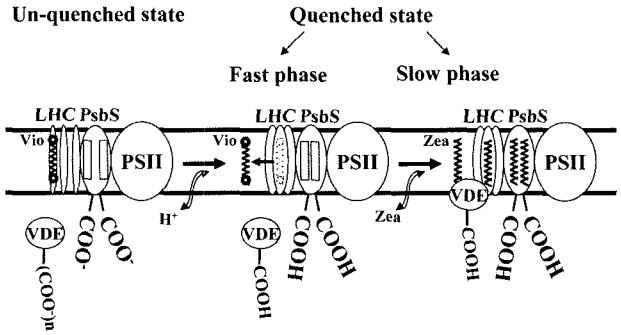

Figure 2. Model of energy-dependent quenching in plants .This model is modified from that presented in [35]. Under excess light, lumenal pH decreases. Lowering of thylakoid lumen pH leads to protonation of PsbS. At low lumenal pH, VDE is also protonated, and the activated VDE can convert Vio released from LHCs to Zea. Zea binding to PsbS and/or LHCs induce the conformational changes. The fast process can be induced within seconds and the next slow phase is completed within few minutes.

Indeed, our understandings of qE has been deepened by a number of investigations on role and function of this process, but many questions remain open and need to be solved, especially with respect to qE-deficient mutant plants (*npq1-2*, *npq4-1*, *pgr* lack in Zea, PsbS and petC, respectively).

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