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Mini Review

On photoprotective mechanisms of carotenoids in light harvesting complex

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ABSTRACT

Carotenoids in light harvesting complex (LHC) play an important role in preventing plants photodamage caused by excess light. Non-photochemical quenching (NPQ) is an important mechanism adopted by plants to deal with high light intensity and the major component is referred to as energy dependent quenching (qE). Despite numerous studies have been devoted to investigating the site and mechanism of qE, there are still much debate on these topics. In this article, we discussed the possible site and underlying mechanism of qE based on the structural similarity of carotenoids. Moreover, being as good antioxidants, carotenoids' potential protective effects against LHC photo-oxidation by quenching active oxygen species or triplet excited state chlorophyll are also discussed.

1. Introduction

Plants use light energy for their metabolism. Solar energy absorbed by major light harvesting complex (LHCIIb) is transferred to photosystem II (PSII) reaction centers to be used in photosynthesis. However, the light in environment often fluctuates greatly, which presents a major challenge to plants. Under high light condition, the excess energy absorbed by plants can cause destructive effect if the photosynthetic apparatus cannot deal with it efficiently. Plants have developed several protective mechanisms to dissipate excess light [1], and the most effective one of them is non-photochemical quenching (NPQ) [2–5]. The major component of NPQ is referred to as energy dependent quenching (qE), which can develop and relax within seconds [2]. Furthermore, plant carotenoids (Fig. 1) are natural antioxidants and their antioxidant capacities may also protect the photosystem from photodamage.

2. Energy dependent quenching

2.1. The qE site

It has been widely accepted that the qE site locates in the antenna system but not in the reaction center [2], while the precise components responsible for qE in PSII are still not well understood [3–5]. The antenna system is composed of the major complex LHCIIb and the minor components CP 24, CP 26, and CP 29. There is no decisive evidence to support whether the major or minor LHCII is the precise site of qE. Ruban and coworkers suggested that the qE occurred in the major trimeric antenna LHCIIb and the quenching center is a low-lying excited state of a carotenoid (lutein 1) [3]. On the other hand, according to Ahn et al. [4], a charge-transfer (CT) mechanism in minor complexes is responsible for NPQ. As to the CT mechanism, a strongly coupled chlorophyll dimer in A5 and B5 site in CP29 can accept an electron from the zeaxanthin in the L2 site [4]. Both the proposed quenching mechanisms occurred in the LHCIIb require a conformational change that switches the complex from the light-harvesting state to the quenched state. It is believed that the trans-thylakoid ΔpH formed in high light can directly or indirectly induce conformational change in light harvesting complex [3,4]. The X-ray structures of spinach and pea LHCIIb have been determined from crystals grown either at pH 5.4 or 7.5, and the root mean square deviation between the Cα coordinates of their LHCIIb is 0.35 Å [5]. Thus, it appears that the LHCIIb structure in acidic pH is very close to that in neutral environment. However, the crystal structure can not represent the physiological state completely. It is observed that the genetic mutant lacking every kind of LHCII does not perform the qE-null phenotype, indicating that qE does not occur in a single LHCII [6,7]. In addition, the *npq4* mutant which lacks the active protein PsbS cannot dissipate excess absorbed light energy at all [8], suggesting that PsbS may be the site of qE [9]. However, recent work shows that plants lacking PsbS protein can reach the same level of NPQ in a time-delayed manner [10], implying that the quenching site is not located in PsbS. Bassi et al. suggested that PsbS controlled the dissociation of the PSII-LHCII complex under excess light [11,12]. In addition, it is recently showed that a structural reorganization involving LHCII dissociation from PSII occurred during NPQ [13]. These results suggest that PsbS may regulate the NPQ process by controlling the macrostructure of PSII-LHCII complex rather than by directly quenching the singlet chl a.

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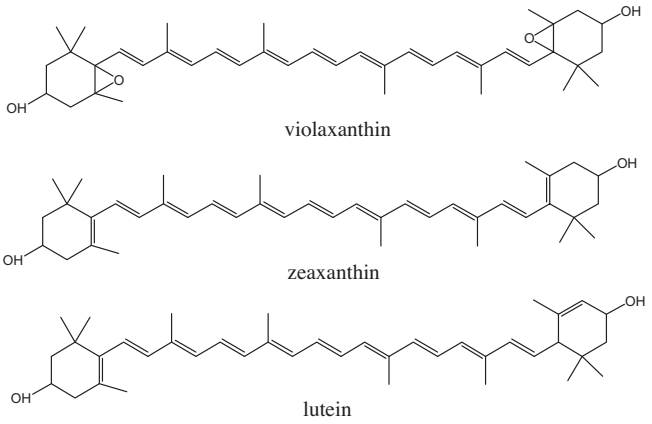

Fig. 1. Chemical structures of violaxanthin, zeaxanthin and lutein.

2.2. What can we learn from the structural similarity of carotenoids

Besides the debates on the precise site of qE, the rule of the xanthophyll cycle in qE also remains unclear. Under excess light condition, the acidic environment activates the xanthophyll cycle that converts violaxanthin to zeaxanthin [14], which strongly enhances NPQ [15]. We can learn the exact function of carotenoid in NPQ from the genetic mutant which lacks an enzyme in the carotenoid biosynthesis pathway [16]. The *npq1* mutant is unable to convert violaxanthin to zeaxanthin, but it still possesses a considerable level of qE, suggesting that the xanthophyll cycle is not a prerequisite of qE [16].

The structures of carotenoids in LHCIIb are very similar, implying that they may potentially have similar function in qE. Besides the *npq1* mutant, the genetic mutant deficient in synthesizing any kind of carotenoids still has a considerable amount of qE [16], which suggests that the carotenoids in LHCII may have functional redundancy. It is interesting to find that the double mutant *lut2npq1*, which loses both zeaxanthin and lutein, has an NPQ-null phenotype [17]. Furthermore, both zeaxanthin and lutein have a ring which has a double bond conjugating with the polyene chain, being a hint that this ring may plays a role in qE. We call this conjugating ring as quenched ring (Q ring, Fig. 2). Indeed, in the minor light harvesting complex Lhcb5, the chl a 613, which is close to the Q ring, is an important facilitator of aggregation-dependent quenching [18]. Also, it is suggested that there is a rotation of the Q ring of lutein 1 in the quenched state by comparing the conformations of lutein 1 and lutein 2 [19]. In addition, it has been found that lutein accumulation in the absence of zeaxanthin can restore NPQ in the *Arabidopsis thaliana npq1* mutant [20]. The underlying reason may be that lutein accumulation can compensate the Q ring loss due to the zeaxanthin deficiency.

2.3. Protein conformational change acts as a switch between the quenched and unquenched state

It has been proposed that the qE was induced by conformational changes in LHCIIb [21]. The LHCIIb can be locked into the quenched or unquenched state by the protein cross-linker glutaraldehyde, implying that a protein conformational change in LHCIIb may be involved in qE [21]. Furthermore, reversible fluorescence red shifts have recently been detected in LHCIIb, which have been interpreted as a result of small conformational changes in the LHCIIb scaffold [22]. In addition to neoxanthhin, chl a and chl b [23], the conformational change in lutein 1 binding domain in LHCIIb has been observed very recently, and the lutein 1 undergoes a twisting process in the quenched state [24]. These results suggest that there

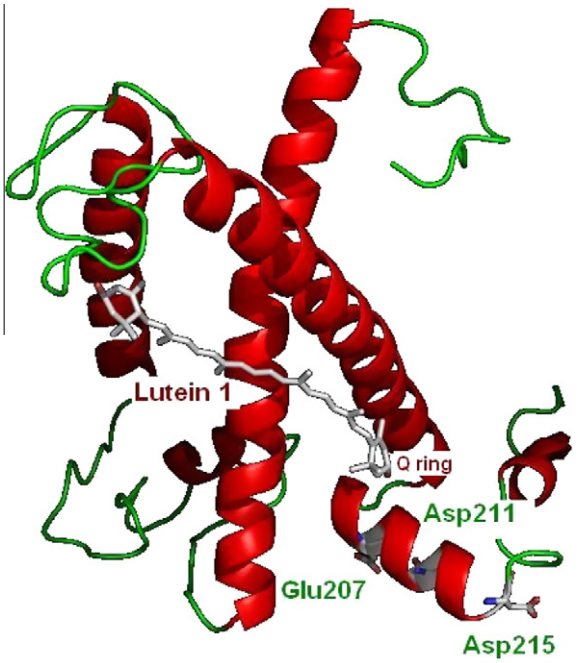

Fig. 2. The lutein 1 domain of LHCIIb (PDB code: 1RWT). Three acidic amino acids (Glu207, Asp211 and Asp215) near the Q ring of the L1 lutein that may respond to the pH are labelled.

is conformational change in LHCIIb during the switch from the unquenched to the quenched state. Furthermore, a thermodynamic model has been employed to describe the transition from the unquenched to the quenched conformation [25]. This model indicates that the quenched state is an intermediate state between the unquenched and the unfolded state, and it is maybe the same domains that promote quenching and unfolding in LHCIIb [25]. On the basis of the refolding experiment of monomeric LHCIIb, it is pointed out that the loop region folding is the last step during LHCIIb assembling [26]. These collective findings support that the loop region may play an important role in qE by inducing conformational change.

Lutein 1 is very essential to the LHCIIb stability [27], and the Q ring of lutein 1 locates in the lumen side of chloroplast where qE occurs due to low pH. The loop near the Q ring of lutein 1 has three acidic amino acids: Glu207, Asp211 and Asp215 (Fig. 2), and the three amino acids may be the key region that responds to the pH change. In excess light, the acidic environment may cause the protonation of the three amino acids, and subsequently induces the conformational change of the Q ring. In summary, the Q ring may act as a switch of qE process and further study is required to explore the underlying molecular mechanism.

2.4. The regulatory effect of protein on the S_1 energy of carotenoids

Besides the light-harvesting function, the S_1 state of carotenoid is believed to play a role in the qE [29]. Employing transient absorption and fluorescence spectroscopy, the S_1 energies of violaxanthin, zeaxanthin and lutein have been determined in organic solvents [28] and in LHCIIb protein environment [29]. The results showed that the S_1 energies of all three carotenoids were slightly below the Q_y state of chlorophylls [28,29]. However, the experimental results may be referred to as the energy of the relaxed S_1 state. Dreuw and coworkers suggested that the S_1 energies of the three carotenoids were all slightly higher than that of the Q_y state of Chl a, indicating that the carotenoids in LHCIIb can not quench the singlet Chl a directly [30]. However, a short-lived Car-Chl ex-

cited state has been observed experimentally by selectively exciting carotenoid and Chl a in the quenched state, which suggests that excitonic interactions may be formed between them [31,32]. According to these findings, the excess energy absorbed by cholorophylls may be quenched directly by carotenoids in LHCIIb. Furthermore, van Grondelle and coworkers [3] reported that the energy transfer from Chl a to the S_1 state lutein involves a mechanism termed as incoherent coupling. This mechanism suggests that the excitation energy hops from one molecule to another while being localized on a single molecule at any time. A coupling parameter has been proposed to quantify the extent of electronic interactions between carotenoid dark states (Car S_1) and chlorophyll (Chl) states by Bode et al. [31]. They observed that there is a linear correlation between the electronic interactions and chlorophyll fluorescence quenching, indicating that the quenching excitonic Car S_1-Chl may be responsible for non-photochemical quenching [31].

3. The antioxidant effect of carotenoids

As discussed above, plants respond to sudden increase in light intensity through NPQ mechanism, but the npq4 genetic mutant that lacks both PsbS protein and qE can survive in high light condition. It is thus rational to infer that some antioxidants may compensate the deficient in qE [33,34]. Being as excellent natural antioxidant, the carotenoids in LHCIIb may play an important function in photoprotection. For example, the mutant of LHCII possessing only one carotenoid (violaxanthin) becomes more sensitive to photobleaching [27]. As it is hard to discriminate the antioxidant and the quenching effect in protecting plants against light stress, the antioxidant contribution of the carotenoids in LHCII remains to be determined.

The antioxidant mechanisms of carotenoids have been reviewed [35] and they may react with oxidants by hydrogen atom transfer, electron transfer, and energy transfer reactions [35,36]. As there exist hydrogen bonds between the protein amino acids and potential hydrogen atom donating groups (hydroxyls) of the carotenoids in LHCIIb, the hydrogen atom transfer reaction may be retarded to a large extent. In addition, although the zeaxanthin and lutein cation radicals, which were generated through electron transfer reaction, have been detected in minor light harvesting complex [4,37], they were not observed in the major light harvesting complex. These results imply that carotenoids in LHCIIb may directly quench the triplet excited state chlorophyll or singlet oxygen by energy transfer mechanism. Indeed, the L1 lutein of the LHCIIb has the specific property of quenching harmful triplet excited state chlorophyll [38], and zeaxanthin becomes more effective in scavenging singlet oxygen after binding to LHCIIb [38]. Furthermore, it has been reported that the antioxidant effects of lutein and zeaxanthin also depend strongly on their binding to LHC proteins [38,39].

To summarize, the region that binds lutein 1 in the luminal side may be an important regulatory domain regulating qE. It is demonstrated that the Trp222 near the domain is critical for trimerization of LHCIIb which is very important for efficient light harvesting [40]. In addition, this region is also near the xanthophyll binding site, indicating that it may be a regulatory region of qE. Furthermore, by comparing the structural similarity of carotenoids in LHCIIb, we tentatively assign this region as a regulatory domain that responds to the pH change which induces qE.

The antioxidant pattern of carotenoids may change greatly due to their binding to the LHCIIb. In the free binding state, they may interact with oxidant by hydrogen atom transfer reaction, electron transfer reaction mechanism. However, the carotenoids binding to LHCII may protect the photosystem mainly by energy transfer mechanism. The triplet excited state chlorophyll and singlet oxygen produced in high light may be quenched efficiently by carotenoids and their quenching capacity may be enhanced by binding to LHCIIb.

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