Research article

Xanthophyll cycle – a mechanism protecting plants against oxidative stress

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Six different xanthophyll cycles have been described in photosynthetic organisms. All of them protect the photosynthetic apparatus from photodamage caused by light-induced oxidative stress. Overexcitation conditions lead, in the chloroplast, to the over-reduction of the NADP pool and production of superoxide, which can subsequently be metabolized to hydrogen peroxide or a hydroxyl radical, other reactive oxygen species (ROS). On the other hand, overexcitation of photosystems leads to an increased lifetime of the chlorophyll excited state, increasing the probability of chlorophyll triplet formation which reacts with triplet oxygen forming single oxygen, another ROS. The products of the light-dependent phase of xanthophyll cycles play an important role in the protection against oxidative stress generated not only by an excess of light but also by other ROS-generating factors such as drought, chilling, heat, senescence, or salinity stress. Four, mainly hypothetical, mechanisms explaining the protective role of xanthophyll cycles in oxidative stress are presented. One of them is the direct quenching of overexcitation by products of the light phase of xanthophyll cycles and three others are based on the indirect participation of xanthophyll cycle carotenoids in the process of photoprotection. They include: (1) indirect quenching of overexcitation by aggregation-dependent light-harvesting complexes (LHCII) quenching; (2) light-driven mechanisms in LHCII; and (3) a model based on charge transfer quenching between Chl *a* and Zx. Moreover, results of the studies on the antioxidant properties of xanthophyll cycle pigments in model systems are also presented.

Keywords: De-epoxidation, Non-photochemical quenching, Oxidative stress, Xanthophyll cycles

Introduction

An imbalance between the generation of different reactive oxygen forms and a biological system's ability to detoxify reactive intermediates or to repair the resulting damage is commonly known as oxidative stress. Chemically, oxidative stress is associated with the increased production of oxidizing species or a significant decrease in the capability of antioxidant defenses and it may occur in all organisms.[1]

Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. Reactive oxygen species (ROS) can even be beneficial, as they are used by the immune system as a way to attack and kill pathogens and play important roles in cell signalling through a process called redox signalling.

However, when the production and consumption of ROS becomes unbalanced, proper cellular redox homeostasis is threatened, which can cause cell death. It is known that even moderate oxidation can trigger apoptosis.[2] A particular destructive aspect of oxidative stress is the production of ROS, which include free radicals and peroxides. Moreover, some of the less reactive of these species can be converted by oxidation–reduction reactions into more aggressive radical species.[3] Minor amounts of ROS are generated by some enzymes such as oxidases through the autooxidation of different molecules. The oxidases known to produce ROS are NADPH oxidase, lipoxygenases, cyclooxygenases, and xanthine oxidase,[4] while molecules entering autooxidation may be of exogenous and endogenous origin like neurotoxin 5-hydroxydecanoate[5] or catecholamines,[6] and many other compounds.

Furthermore, the ability to accept and donate a single electron by metals such as iron, copper, chromium, vanadium, and cobalt is the basis of reactions that produce reactive radicals and ROS can be generated. The most important reactions are probably Fenton's reaction and the Haber–Weiss reaction, in which a hydroxyl radical (OH) is produced from

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reduced iron and hydrogen peroxide (H_2O_2). However, the major fraction of ROS in living organisms is produced by electron-transport chains, endoplasmic reticulum, plasmatic, and nuclear membranes.[7] In plants ROS are additionally generated during photosynthesis as well as photorespiration[8] and by cell wall–bound NAD(P)H oxidases–peroxidases.[9]

The exposure of plants to stresses such as high-light stress, ultraviolet radiation, drought stress and desiccation, salt stress, chilling, heat shock, heavy metals, air pollutants, mechanical stress, nutrient deprivation, and pathogen attack can give rise to an excess accumulation of ROS at the cellular level.[10] Since ROS can be viewed as cellular indicators of stress and as secondary messengers involved in the stress-response signal transduction pathway, their level has to be kept under tight control by ROS scavenging mechanisms, including enzymatic and non-enzymatic antioxidants (Fig. 1).[11]

One group of non-enzymatic antioxidants that exists in plants is the products of de-epoxidation occurring in the processes generally called the xanthophyll cycle.

Xanthophyll cycle protects plants against oxidative stress generated by high-light intensity

The regulation of excitation density in the photosynthetic apparatus is particularly important under high-light conditions, owing to the risk of the light-induced generation of ROS, leading to the photodegradation of the photosynthetic apparatus. Overexcitation conditions on the one hand lead to the over-reduction of the NADP pool and to superoxide ($O_2^{-\cdot}$) production in the chloroplast by Mehler's reaction.[12] $O_2^{-\cdot}$ can subsequently be converted to H_2O_2 or a $^{\bullet}OH$.[13] On the other hand, overexcitation of photosystems leads to an increased lifetime of an excited state of chlorophyll (Chl), chlorophyll single formation (^{1}Chl*), increasing the probability of a Chl *a* triplet formation (^{3}Chl*) which reacts with oxygen ($^3O_2$) forming singlet oxygen ($^1O_2$), ROS (Fig. 2(A)). Thus, photosystem II (PSII) and light-harvesting complexes (LHCII) localized at the periphery of each PS become an important source of $^1O_2$.[14] LHCII comprises Chl *a* and *b* (eight and six molecules per protein monomer, respectively), four molecules of xanthophylls (two molecules of lutein (L), one neoxanthin (Nx), and one violaxanthin (Vx))[15] appear in a trimeric form in its native state.[15]

One of the most efficient mechanisms protecting plants and other photosynthesizing organisms under overexcitation conditions is the xanthophyll cycle. At the molecular level some carotenoids of this cycle act as quenchers of ^{1}Chl*, thus preventing the formation of ROS (Fig. 2(B)).[16,17]

Six types of xanthophyll cycle have been described. Four of them are based on beta-xanthophylls and two on alpha-xanthophylls (Table 1).[18] All xanthophyll cycles have in common the light-dependent transformation of epoxidized xanthophylls to de-epoxidized ones in high light, which facilitates the dissipation of excitation energy, and their reversion to epoxidized xanthophylls in low light.[16,19] This dissipation is obtained by mechanisms collectively referred to as non-photochemical quenching (NPQ). The predominant NPQ component is induced as a result of the acidification of the thylakoid lumen associated with the formation of the chloroplast proton motive force

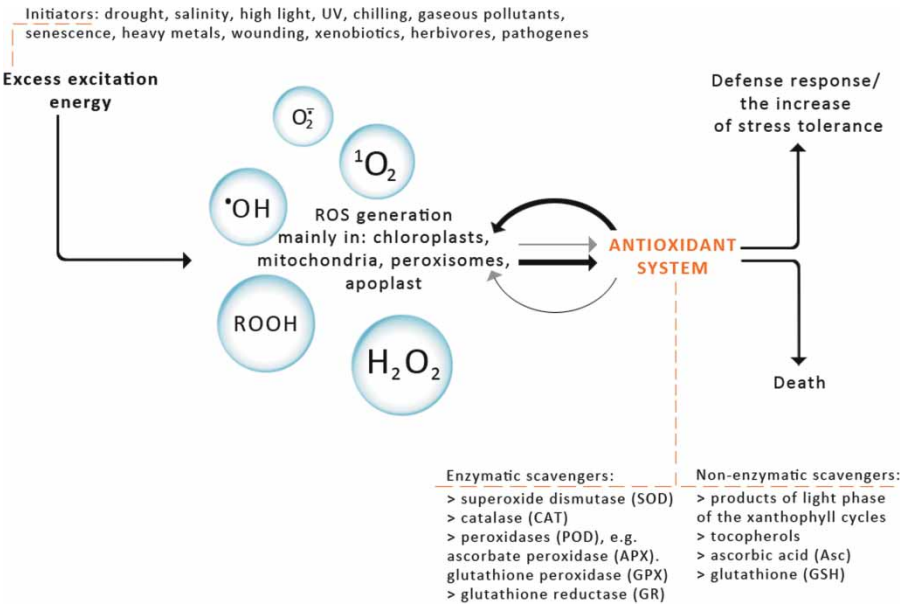


Figure 1 The response of plants to different abiotic and biotic stresses. The antioxidant system (enzymatic and non-enzymatic scavengers) enables plants to regulate ROS level and influence on ROS-dependent signal induction. High ROS generation and weak interaction of the antioxidant system cause cell death. Domination of antioxidant system over ROS generation allows defence response or increase in stress tolerance.

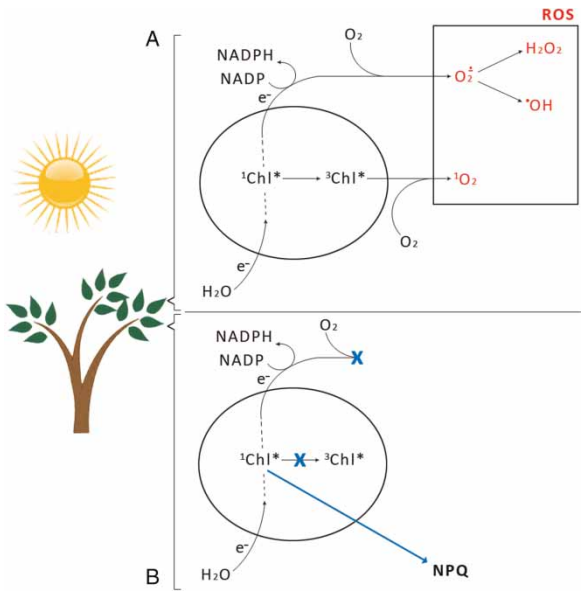


Figure 2 Routes of generation of ROS during the light phase of photosynthesis. ^{3}Chl* on account of increasing chlorophyll excited state (^{1}Chl*) in incomplete photochemical quenching, reacts with oxygen ($^3O_2$) to form $^1O_2$. Over-reduction of the NADP pool causes O_2^- production by Mehler's reaction (A). The photoprotective mechanism of excess energy dissipation through NPQ, blocking generation of ROS (B).

and defined as energy quenching (qE).[20] In addition to qE, relaxing within 2–5 minutes, a slowly relaxing component of the NPQ process is known as qI (photoinhibitory quenching), with a half-time of approximately 30 minutes and longer (depending on the degree of photoinhibition).[21] A third quenching component (qT), relaxing within 15–20 minutes, also has been reported.[21,22]

The most commonly occurring type of the six xanthophyll cycles and most intensively studied is the Vx-cycle, also called the xanthophyll cycle, where the main product of strong light-stimulated de-epoxidation is zeaxanthin (Zx). Thorough and detailed studies by different research groups have shown a dependence between the content of Zx and NPQ in chloroplasts.[23] An even better correlation was found between NPQ and the total amount of Zx and antheraxanthin (Ax).[24] An increase in NPQ after high-light treatment and its correlation to Vx de-epoxidation in spinach (*Spinacia oleracea*) leaves, isolated chloroplasts, and purified LHC complexes have also been observed.[25] Similarly, in diatoms, the NPQ level was well correlated with the diatoxanthin (Dtx) amount, which was created during the de-epoxidation of diadinoxanthin (Ddx) (Table 1).[25,26] In other experiments, a photoprotective action of Dtx during prolonged UV-A and UV-B illumination of diatoms (*Thalassiosira weissflogii*) has been demonstrated.[27] These UV-insensitive diatoms shown increased activity of the Dtx cycle as a response to light stress.

Studies of mutants of the green algae *Chlamydomonas* have also shown that L, like the de-epoxidation products of Vx or Dtx, has a significant role in energy dissipation.[26]

It also has been observed that the de-epoxidation of lutein-epoxide (Lx) to L (Table 1) facilitated the rapid engagement of NPQ, and that this process may be fine-tuned by concurrent Zx accumulation inducing strong energy dissipation in plants having both an Lx-cycle and Vx-cycle.[18]

On the other hand, photoconversion of siphonaxanthin (Sx) to L (Table 1) detected in the green alga *Caulerpa racemosa*, showing significant relationships to Vx–Ax interconversion, suggests a similar activation signal for these two mechanisms. In particular, both Ax and L reached their highest values not only under high light, but also at sunrise when light intensity was lower.[28] This last feature suggests a similar and very high sensitivity of the two cycles to light and a photoprotective role for the interconversion between Sx and L may be hypothesized, even if this hypothesis needs to be tested through adequate studies. Also, in favour of this hypothesis are the biochemical similarities between L and Ax reported in the literature, the energetic state analysis revealing similar S_1 values for Ax and L (Fig. 3).[28]

All these observations show that all the products' xanthophyll cycles that are created under light conditions are effective quenchers of ROS.

It is known that carotenoids possess at least two spectroscopically important low-lying excited states denoted S_1 and S_2,[29] with S_0 indicating the ground state. Electronic transitions between S_0 and S_1 are forbidden because these two states have the same (A_g) symmetry in the idealized C_{2h} point group. Electronic transitions to and from S_1 and S_0 are forbidden by symmetry, but such a transition is allowed between S_0 and S_2 because S_2 possesses B_u symmetry. Vx, Zx, and all xanthophylls display very strong absorption in the visible region. This absorption is associated with an electronic transition between S_0 and S_2. This higher energy state is denoted 1^1Bu. Although there is some evidence that additional A_g states lie near the S_1 (2^1A_g) and the 1^1Bu states in long polyenes and carotenoids,[30] the latter is usually referred to as S_2 because it is the most easily observed and spectroscopically accessible state above S_1.

Direct quenching of overexcitation by products of the light phase of xanthophyll cycles

A key factor in evaluating the efficiency of the mechanisms that were proposed to explain NPQ was the energy of the S_1 states of the epoxy- and de-epoxyxanthophylls.

It was postulated that the xanthophylls created as an effect of light-induced enzymatic conversion (Table 1; Ax, Zx, L, and Dtx) are the pigments that possess a longer conjugated double-bond system, as compared

to their oxidized derivatives (11 conjugated double bonds for Zx versus 9 for Vx). This fact implies that the lowest-excited singlet state (S_1) of these pigments is located at a lower level on the energy scale with respect to the Q_y level of Chl *a* and suggests a possible quenching of Chl *a* excessive singlet excitation by these xanthophylls but not by their oxidized derivatives (Fig. 3).[31]

One of the hypotheses explaining the role of xanthophyll cycles in NPQ assumes a direct quenching of overexcitation by the de-epoxyxanthophylls created under high-light conditions. This model proposes that a downhill energy transfer from Chl *a to* Zx, Ax, Ddx, or L occurring after a pH-activated structural change in the pigment–protein complex has facilitated the energy exchange.[32,33] This idea has gained support from estimates of the energies of the lowest-lying singlet (S_1) states of the xanthophylls from either the dynamics of these states or the fluorescence of a series of shorter carotenoids (less than 10 carbon–carbon double bonds), and extrapolation of the energies to the longer molecules including the pigments involved in the xanthophyll cycles.[33] Spectroscopic and kinetic investigations have revealed that the energies of the S_1 states of the xanthophyll pigments are low enough to quench Chl excited states.

It was previously published that a clear relationship exists between the carotenoid S_1 energy level and its ability to quench Chl fluorescence.[28] The use of carotenoids with S_1 energies above that of Chla had little, if any, effect on NPQ. A carotenoid molecule may be considered as a 'quencher' or 'non-quencher' depending whether the S_1 energy is below or above that of Chl *a*, respectively.

Model system studies using liposomes with embedded LHCII and xanthophyll cycle pigments revealed that the xanthophylls decrease the relative quantum yield of Chl fluorescence, quenching the Chl via singlet excitation transfer, Zx being a better quencher than Vx.[34]

The ability of five carotenoids (Zx, Vx, L, Nx, and beta-carotene) to quench Chl *a* fluorescence was also tested by trapping both types of pigments in micelles of triton X-100. Among the xanthophyll cycle pigments studied, only Zx was a good quencher of Chl *a* fluorescence, comparable in its efficiency to that of beta-carotene. Vx was a much weaker quencher than Zx. L and Nx actually enhanced the fluorescence. Moreover, it was also demonstrated that the Zx quenching ability was related to Zx dimer formation immediately on addition of this pigment to the Chl-containing micelles. It has been postulated that this dimerization may play a role in Zx functioning in the photosynthetic apparatus.[35]

It also should be mentioned that some of the recent determinations of the energy levels of the xanthophyll cycle pigments show that the difference between the energies of the S_1 state of Vx and Zx is very small, and therefore essentially different Chl excitation quenching efficiencies for these two compounds may not be expected.[36] Accordingly, no exceptional Chl singlet excitation quenching has been observed in the antenna complexes isolated from the L mutants of *Arabidopsis thaliana*, in which L was replaced with Zx.[17,37]

Moreover, it was also found that the S_1 level of not only Zx but also Vx lies below the Q_y level of Chl.[38] This has significant implications for the mechanism of xanthophyll cycle photoregulation in plants. A mechanism involving direct quenching via singlet–singlet energy transfer seems to be invalid. According to these data, Vx could even be a more efficient quencher than Zx, because its S_1 level lies below but closer to the Chl Q_y transition than the S_1 level of Zx. In this respect, an aggregation model based on the indirect participation of the xanthophyll cycle carotenoids in the process of photoprotection is a more promising candidate to explain the function of the xanthophyll cycle in the quenching of excess energy in the antenna complexes of higher plants.[38]

Three main models for excess energy dissipation by xanthophyll cycle products created under light conditions have been proposed.

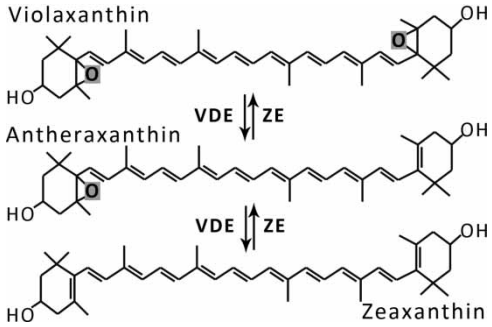
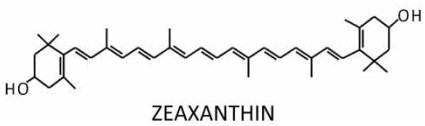
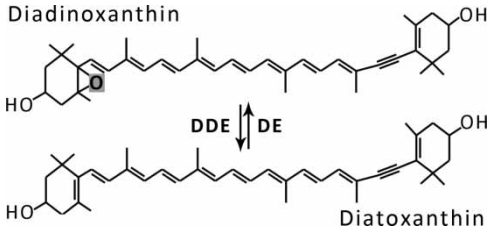
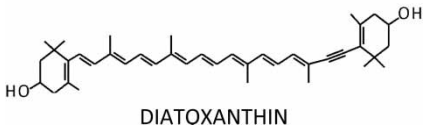
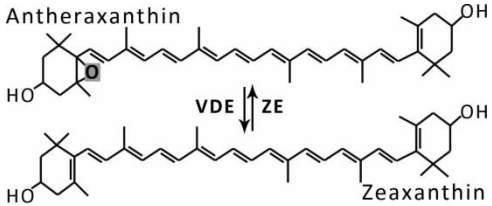
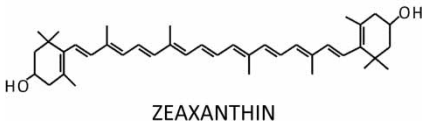
I. LHCII aggregation-dependent indirect quenching of overexcitation

According to this model, qE occurs upon aggregation of the major, trimeric LHCII complex of PSII. This produces a conformational change within the protein and promotes energy transfer from Chl *a* to S_1 excited state of L bound to the LHCII (Fig. 4).[39,40]

LHCII consists of six types of monomeric proteins called as Lhcb which fall into two groups with respect to the sites where they bind L and Vx or Zx. The first group includes Lhcb1, Lhcb2, and Lhcb3, the components of the major trimeric LHCII, binding L at sites L1 and L2, while Vx or Zx bind at site V1.[15,41] The second group of Lhcb proteins includes monomeric Lhcb4, Lhcb5, and Lhcb6, which bind L at site L1 and Vx or Zx at site L2.[42,43] These proteins do not have a V1 site and thus exchange Vx with Zx in site L2,[44] while Lhcb1-3 do not.

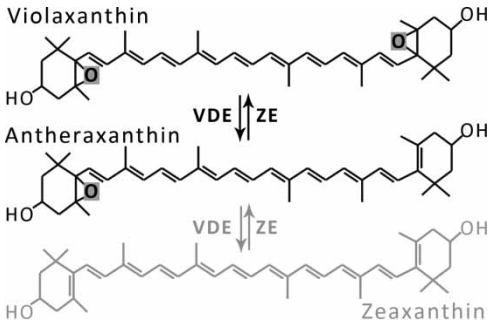
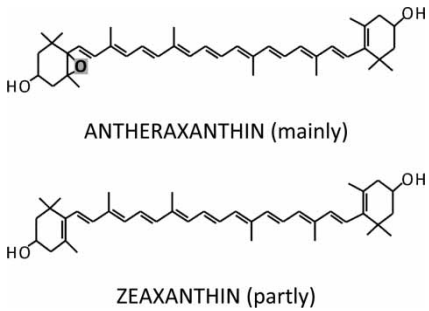
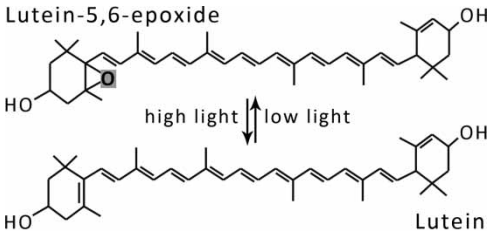
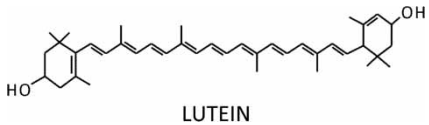
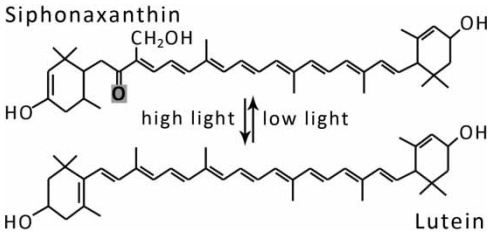
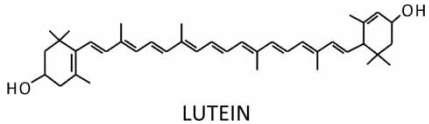
Recently, an aggregation-dependent LHCII quenching model was supported by the observation of a red-shifted fluorescence lifetime component both in aggregated LHCII trimers binding Zx and in quenched leaves.[45] Zx bound at site V1 of LHCII acts as an allosteric modulator of L-dependent quenching,[45] whereas aggregation *in vitro* has been proposed to entrain an intrinsic conformational transition in the LHCII complex, responsible for the establishment of the quenching reaction (Fig. 4).[46] Carotenoid S_1-Chl excited state coupling was recently measured in

Table 1 Types of xanthophyll cycles

Type of xanthophyll cycle		Products of light phase of the xanthophyll cycles	Description
Violaxanthin cycle (xanthophyll cycle)			*Enzymes* • Violaxanthin de-epoxidase (VDE) • Zeaxanthin epoxidase (ZE) *Occurrence* • Higher plants • Mosses • Lichens • Algae (phaeophyta, chlorophyta, rhodophyta)
Diadinoxanthin cycle			*Enzymes* • Diadinoxanthin de-epoxidase (DDE) • Diatoxanthin epoxidase (DE) *Occurrence* • Algae (diatoms, phaeophytes, dinophytes, haptophytes)
Antheraxanthin cycle			*Enzymes* • Violaxanthin de-epoxidase (VDE) • Zeaxanthin epoxidase (ZE) *Occurrence* • *Gracilaria gracilis* • *Gracilaria multipartite*

Continued

Table 1 Continued

Type of xanthophyll cycle		Products of light phase of the xanthophyll cycles	Description
Xanthophyll cycle in *Mantoniella squamata*			*Enzymes* • Violaxanthin de-epoxidase (VDE) • Zeaxanthin epoxidase (ZE) *Occurrence* • *Mantoniella squamata*
Lutein epoxide cycle			*Enzymes* • Violaxanthin de-epoxidase (VDE) • Zeaxanthin epoxidase (ZE) *Occurrence* • Leaves of 62% of the 188 tested species
Siphonaxanthin cycle			*Enzymes:* no data available *Occurrence* • *Caulerpa racemosa*

Note: Non-specific Xanthophyll cycle – siphonaxanthin is not epoxy xanthophyll.

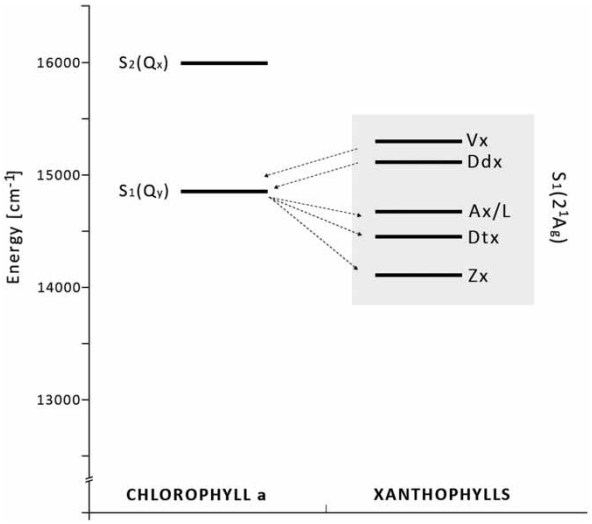


Figure 3 Energy-level diagram. The localization of the S_1 and S_2 energy levels of chlorophyll *a* (Chl *a*) and S_1 energy levels of carotenoids. The energies of Chl *a*: S_2(Qx) 16 000/cm and S_1(Qy) 14 700/cm. The energies of the S_1 state of xanthophylls: violaxanthin (Vx, 15 290/cm), diadinoxanthin (Ddx, 15 130/cm), antheraxanthin and lutein (Ax/L, 14 720/cm), diatoxanthin (Dtx, 14 485/cm), and zeaxanthin (Zx, 14 170/cm).[28] Arrows from left to right represent forward energy transfer (light-harvesting); arrows from right to left – reverse energy transfer (NPQ).

isolated LHCII complexes and correlated with the NPQ amplitude *in vivo* in different mutants such as *n*pq1, *n*pq2, *lut2*, and *PsbS* over-accumulating lines.[47]

Criticisms of this model have been raised on the basis that:

1. the effect of down regulating the components of LHCII *in vivo*, namely Lhcb1+2[48] or Lhcb3,[49] is, at best, very small;
2. quenching and other spectral changes attributed to LHCII occur in Lhcb4 and Lhcb5 as well, even more promptly than in LHCII,[50]
3. L cannot be the only quencher during NPQ since the *lut2n*pq2 mutant having Zx as the only xanthophyll is active in NPQ as well as the L-less mutant *lut2*.

II. Light-driven reactions in LHCII as a mechanism of the indirect quenching of overexcitation by products of the light phase of xanthophyll cycles

This model is postulated because under strong light illumination both photo-isomerization of *all-trans* Vx bound to LHCII to the *cis* isomer and a light-induced trimer to monomer transition in LHCII have been observed (Fig. 5).[51,52] A very recent examination of the molecular organization of LHCII, based on fluorescence lifetime imaging microscopy, revealed that *all-trans* Vx stabilizes the trimeric organization of the complex, in contrast to Zx which promotes a monomeric state of LHCII. The rate of excitation energy transfer from Vx to Chl s in LHCII is extremely low[41] and therefore the light energy absorbed by Vx may be utilized to drive the isomerization of the pigment to the *cis* isomer and to cause a trimer to monomer transition in LHCII, which then leads to a reduction in the Chl fluorescence lifetime (Fig. 5). The shortening of the Chl fluorescence lifetime reflects a more efficient singlet excitation thermal dissipation and therefore the light-dependent process is discussed in terms of photoprotective activity within LHCII. Moreover, the operation of the xanthophyll cycle in the photosynthetic apparatus requires Vx to be freely available within the lipid phase of the thylakoid membrane for de-epoxidation to Zx.[43] Vx is a xanthophyll relatively weakly bound to the protein environment of LHCII, and the process of the light-driven change of this pigment's molecular configuration can result in its uncoupling from the protein and its transfer to the lipid environment of the membrane. Certainly, light-dependent LHCII monomerization makes it easier, or even possible, for Vx to migrate from the protein to the lipid environment. Vx in an *all-trans*, fully relaxed configuration is a specific substrate of the de-epoxidase enzyme[53] and the pigment tends to adopt such a configuration after light-driven transformation, due to the energy minimization process.[54]

III. Model of charge transfer quenching between Chl *a* and Zx

The assumption that NPQ is connected with the formation of a charge-transfer (CT) state between Chl *a* and Zx has been proposed on the basis of quantum chemical calculations[55] and ultra-fast pump–probe experiments on isolated thylakoid membranes.[56] The CT mechanism involves energy transfer from bulk Chl molecules to a Chl–Zx heterodimer that undergoes charge separation followed by recombination, thereby transiently producing a Zx radical cation ($Zx^{+\cdot}$) with a very short relaxation time (50–200 ps), as expected of an efficient quencher (Fig. 6). The formation of $Zx^{+\cdot}$ in thylakoids depends on the three components needed for qE *in vivo*: lumen acidification, PsbS activation, and Zx production.[56,57]

Signals from $Zx^{+\cdot}$ formation have been found in isolated monomeric Lhcbs, but not in LHCII.[57–59]

The mutation analysis of Chl binding sites in Lhcb4[58] showed that for CT quenching a Chl pair called Chl A5 and Chl B5 is critical rather than a single Chl *a* chromophore. The involvement of a Chl pair is reasonable since charge delocalization over the Chl pair would stabilize the CT state. Chl A5–B5 are located in the proximity of the L2 domain while Zx binding to this site induces a conformational change bringing Chl A5 into excitonic interaction with Chl B5, switching the protein to a dissipative state by $Zx^{+\cdot}$ formation. Also, Lhcb6 antenna complexes show a $Zx^{+\cdot}$ formation, while in the Lhcb5

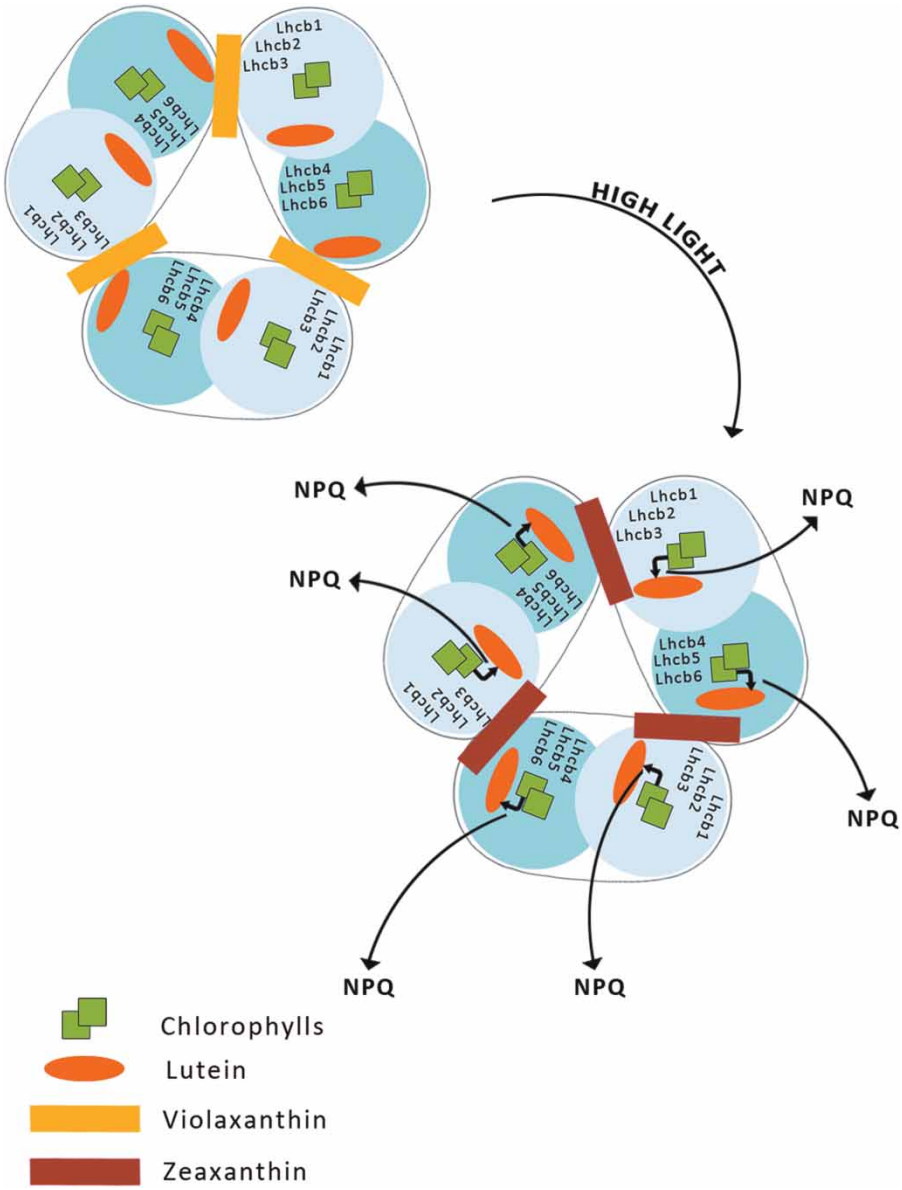


Figure 4 Aggregation-dependent indirect quenching of overexcitation by LHCII. The conformation change produces energy transfer from Chl *a* to a lutein.

complex two distinct CT quenching sites were detected involving, respectively, Zx and L radical cation species, depending on Zx binding to the L2 binding site. Thus, Zx in site L2 acts both as a quencher and as an allosteric modulator of L CT efficiency into site L1.[59] L radical cation was also recently detected in Lhcb6 and Lhcb4 complexes binding L as the only xanthophyll.[60]

Criticism of this model is connected with:

1. a double mutant lacking both Lhcb5 and Lhcb6 and reduced in Lhcb4 retains most NPQ activity;
2. the low level of minor complexes undergoing CT quenching *in vitro* (~1%) versus *in vivo* (30%), implies the presence of factors, possibly PsbS, ΔpH or interactions with protein partners, which stabilize the dissipative conformation[59] *in vivo*;
3. LHC protein conformational change induced by interaction with PsbS has not been reproduced *in vitro*, so far;
4. the relation between CT quenching and the S_1 population is seen as a consequence of charge recombination on carotenoid radical cation formation.

The interaction of Zx with LHCII via PsbS was also postulated as an explanation of the fact that LHCII is involved in the catalysis of NPQ, despite the fact that LHCII trimers containing $Zx^{+\cdot}$ could not be found in solution.[57,60] Moreover, PsbS was not found to bind Zx with LHCII either in *in vivo* or *in vitro* studies.[61]

The xanthophyll cycle is a mechanism protecting plants against oxidative stress not only in the pigment–protein complexes

A very important aspect of the operation of the xanthophyll cycle in the thylakoids as a mechanism protecting plants against oxidative stress is the direct presence of carotenoid pigments in the lipid phase of

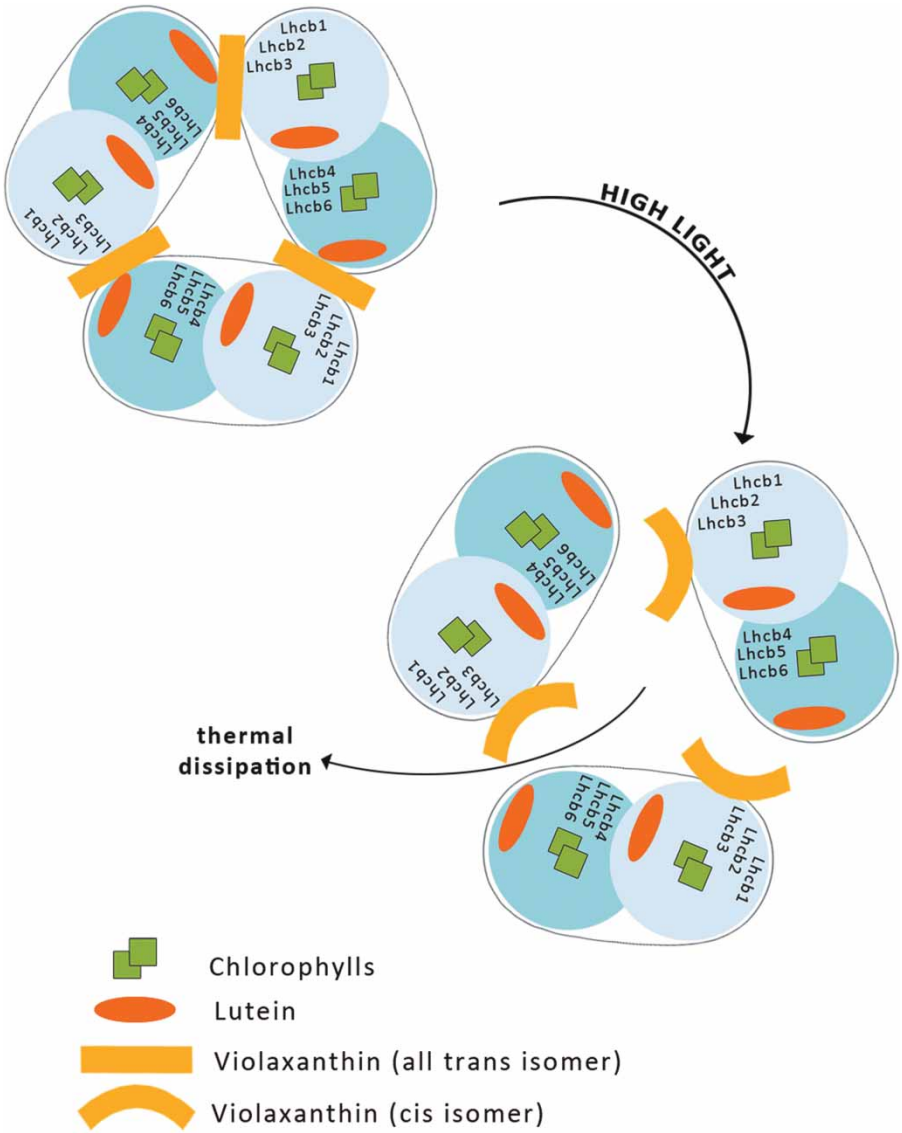


Figure 5 Model of light-induced transformation of the antenna complex LHCII. Illumination in physiological conditions results in Vx all-*trans* to Vx *cis* isomerisation, which causes dissociation of LHCII trimers to monomers, resulting in increased thermal energy dissipation.

the thylakoid membrane and not assembled into pigment–protein complexes.[17,62,63]

The effect of Zx on lipid degradation under strong light conditions was observed in pea leaves.[64] The content of lipids in leaf cells decreased and the saturated/unsaturated lipid ratio increased. Lipid degradation was more significant when Zx formation was inhibited by dithiothreitol (DTT).[65] Similar results came from experiments in which lipid content was measured in response to high illumination in the *n*pq1 mutant, deficient in the production of Zx.[65] In comparison with the wild *Arabidopsis* form, the *n*pq1 mutant had a significantly higher level of lipid photooxidation. Interestingly, in tomato leaves, the Zx level and lipid degradation (measured as ethylene formation) were also correlated. At 3°C and under high light (low level of created Zx), ethylene production was intensive. But at 23°C and in high light, ethylene secretion was lower and the Zx content increased.[66]

The antioxidant properties of Zx were also tested in model systems. It was observed that Zx was the most effective against oxidation initiated both in the aqueous and lipid phases of all tested carotenoids such as beta-cryptoxanthin, beta,beta-carotene, astaxanthin, canthaxanthin, and lycopene. In a homogeneous organic solution, all tested carotenoids ameliorated lipid peroxidation. Zx, as well as beta, beta-carotene, reacted with ROS at similar rates, giving a similar degree of protection in an organic solution. The reactivity and protective efficiency of the astaxanthin and canthaxanthin were lower.[67] Also L in model systems reacted rapidly with oxidizing agents and was recognized as an important antioxidant factor.[68]

These results point to the significance of the xanthophyll cycle pigments in direct protection of the photosynthetic apparatus against ROS. Although Zx and Vx are normally bound to the antenna proteins, they must

be liberated from their binding sites to the lipid domains surrounding the antenna complexes so as to be accessible as substrates for the xanthophyll cycle enzymes.[69] Thus, significant proportions of Zx and Vx are transiently present in the lipid phase, where they may directly quench ROS.

It was also postulated that Vx-cycle and particularly Zx play a role in senescence, as a photoprotectant against lipid photooxidation.[70] It was also observed that the level of L increased gradually during the aging of primary cabbage leaves while the level of Lx was decreased, although the correlation of this phenomenon with lipid peroxidation was not tested.[71]

Notwithstanding differences between authors, the Vx-cycle is recognized as one of the main adaptation mechanisms responsible for a fast response to peroxidation and for the creation of antioxidant substances in thylakoid membranes that can quench singlet oxygen[72] and other free radicals.[73]

It was also observed that some stress factors like drought,[74] salt stress[75], or chilling stimulate the production of Zx.[76] It is commonly suggested that this is due to xanthophyll cycle activity which plays an important role in protecting the photosynthetic apparatus from photoinhibitory damage under a variety of stressors.

Another aspect of the xanthophyll cycle in protection against oxidative stress was postulated when the effect on the xanthophyll cycle of short-term ozone pollution at high doses under photoinhibitory conditions was studied. The plants were also subjected to direct treatment with H_2O_2, $O_2^{-\cdot}$, and to paraquat as a herbicide.

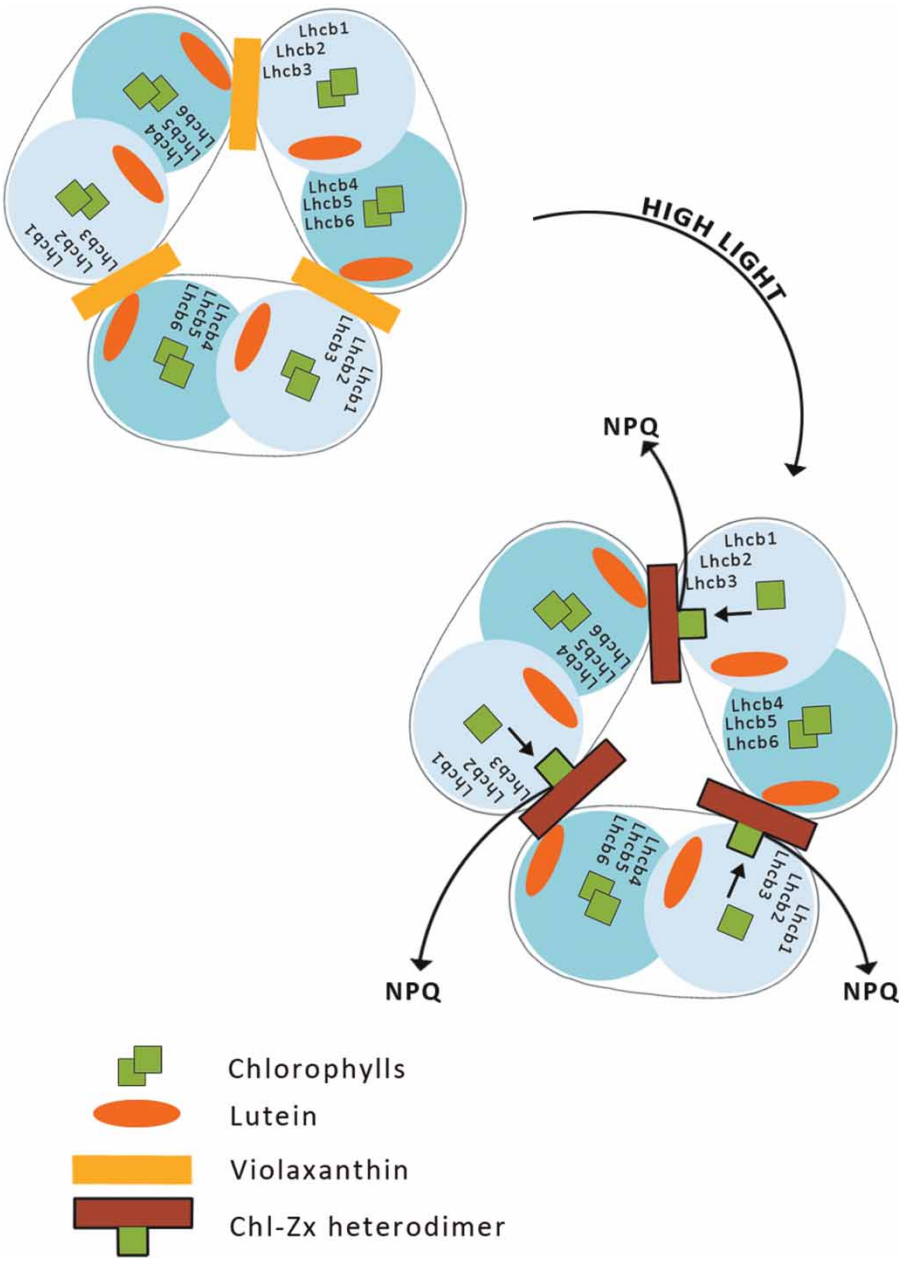


Figure 6 Model of charge transfer quenching between Chl *a* and Zx. This mechanism involves energy transfer from chl to Chl–Zx heterodimer.

Although a degradation of Vx was observed in these experiments, it was not compensated for by the sum of Ax + Zx. It was hypothesized that, under photoinhibitory conditions combined with strong oxidative stress, Vx is used in large part not for the xanthophyll cycle reaction but for the synthesis of growth inhibitory substances such as abscisic acid (ABA).[77,78]

It was documented[79] that Vx is one of the intermediate products in ABA synthesis. One may suppose that conditions causing an increase in Vx de-epoxidase activity would cause a decrease in ABA production. Exogenously added ABA, which inhibits its synthesis, resulted in a higher concentration of Zx and greater photoprotection of PSII.[80]

Conclusion

All of the six types of the xanthophyll cycle are engaged in antioxidant defence in plant cells. Products of the light-dependent phase of these cycles play an important role in the protection against oxidative stress generated not only by excess of light but also by other ROS-generating factors like drought, chilling, heat, senescence, or salinity stress. It was demonstrated that these products are effective quenchers of ROS. Several molecular mechanisms are presented to explain the protective role of the xanthophyll cycle pigments. Some of them refer to direct quenching of ROS and others are based on facilitation of the energy dissipation in the photosynthetic apparatus, which results in decrease in production of singlet oxygen and other free radicals under overexcitation conditions. The mechanisms explaining the protective role of xanthophyll cycles in oxidative stress based on the indirect participation of the de-epoxidized pigments include: (1) quenching of overexcitation by aggregation-dependent LHCII quenching; (2) light-driven mechanisms in LHCII; and (3) charge transfer quenching between Chl *a* and Zx.

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