Chapter 3

How Does Chloroplast Protect Chlorophyll Against Excessive Light?

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Additional information is available at the end of the chapter

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Abstract

Chlorophylls (Chls) are the most abundant plant pigments on Earth. Chls are located in the membrane of thylakoids where they constitute the two photosystems (PSII and PSI) of terrestrial plants, responsible for both light absorption and transduction of chemical energy via photosynthesis. The high efficiency of photosystems in terms of light absorption correlates with the need to protect themselves against absorption of excess light, a process that leads to the so-called photoinhibition. Dynamic photoinhibition consists of the downregulation of photosynthesis quantum yield and a series of photo-protective mechanisms aimed to reduce the amount of light reaching the chloroplast and/or to counteract the production of reactive oxygen species (ROS) that can be grouped in: (i) the first line of chloroplast defence: non-photochemical quenching (NPQ), that is, the dissipation of excess excitation light as heat, a process that takes place in the external antennae of PSII and in which other pigments, that is carotenoids, are directly involved; (ii) the second line of defence: enzymatic antioxidant and antioxidant molecules that scavenge the generated ROS; alternative electron transport (cyclic electron transport, pseudo-cyclic electron flow, chlororespiration and water-water cycle) can efficiently prevent the over-reduction of electron flow, and reduced ferredoxin (Fd) plays a key role in this context.

Keywords: antioxidant, carotenoids, excess excitation energy, non-photochemical quenching, photosystem

1. Introduction

Pigments in plants, cyanobacteria, algae and photosynthetic anoxygenic bacteria are the most important molecules involved in photosynthesis, the only biological process that tunnels

energy on Earth. Pigments play two key roles in photosynthesis: they absorb sunlight and transduce it into chemical energy. The most important pigment is certainly chlorophyll (Chl), an organic compound that typically shows chlorine, a cyclic tetrapyrrole ring, coordinated to a central atom of magnesium (**Figure 1**). This molecular structure is very similar to that found in the eme group in which the central atom is iron. Diversification of various Chls is due to the different side chains bonded to the chlorine ring (Chl *a*, *b*, *c*, *d*, *e* and *f*).

The process of light absorption consists of a sequence of photophysical and photochemical reactions that are subdivided into three stages: (i) light absorption, (ii) utilization of this energy to synthesize ATP and reducing power, reduced ferredoxin (Fd) and NADPH and (iii) absorption and reduction of atmospheric CO_2 into carbon skeleton. However, the most important and true light reaction is represented by charge separation that occurs at the reaction

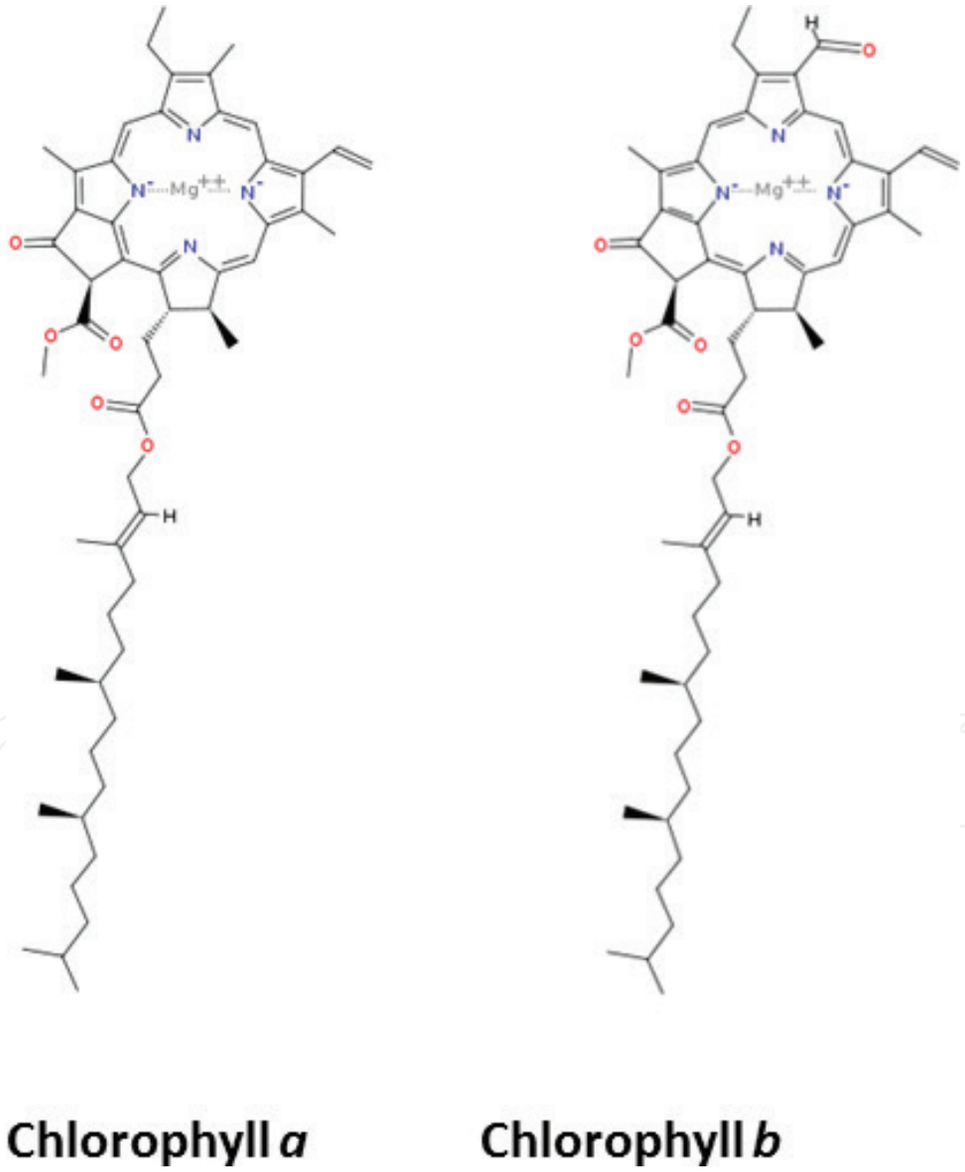

Figure 1. Structures of the chlorophyll molecules.

centres. The process is possible for the presence of organic molecules able to capture sunlight and transduce it in chemical energy namely photosynthetic pigments and that is chlorophylls and, carotenoids. These pigments aggregate with proteins and act as an antenna harvesting the energy of sunlight and tunnelling this energy into the reaction centres located in photosystems. In plants and algae, there are about 200–400 light harvesting molecules. Light harvesting complexes have evolved many adaptive mechanisms that permit photosynthetic organisms to thrive in different environments. The spectral distribution of sunlight that reaches our planet largely covers the absorption spectra of photosynthetic pigments utilized in light harvesting antennas (**Figure 2**). In a general way, light harvesting antennas have developed the ability to optimize light capture under both low- and high-intensity light conditions [1].

The optimal absorption wavelength range for light harvesting antennas is in the red region (680–690 nm), where the energy is utilized by chlorophyll to split water and reduce ferredoxin. The evolution of the most abundant pigments, chlorophyll *a*, is probably related to its efficient absorption in this region in addition to, perhaps, its chemistry and for its redox potential.

All photosynthetic pigments show a chromophore, which possesses two orbitals whose difference in energy falls within the light spectrum. In consequence, a photon of incident light is able to excite an electron from its ground-state orbital to the excited state. From a chemical point of view, the chromophore exists as conjugated π-electron systems or metal complexes. In a conjugated π system, electron excitation occurs between π orbitals spread across alternating single and double bounds (e.g., carotenoids). The metal complex chromophores share d orbitals between transition metals and ligands (e.g., chlorophylls). Really, in the antenna pigments, chromophores are not individual entities, and they synergically interact with each other and this interaction plays a crucial role in the light harvesting mechanism.

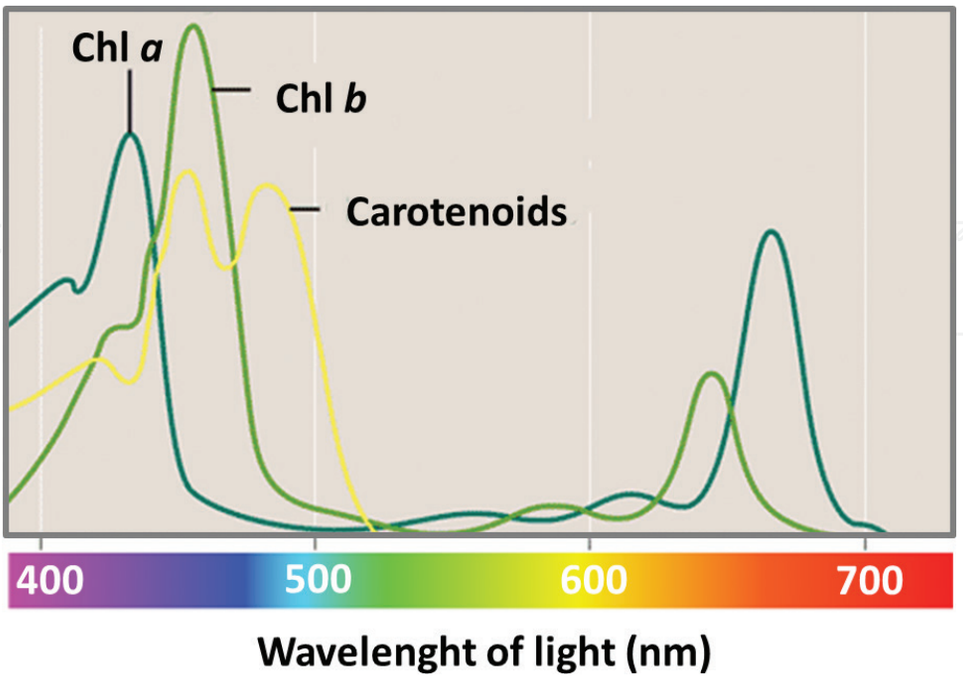

Figure 2. Chlorophyll *a*, *b* and carotenoids absorbance spectra.

Light-harvesting complex (LHC) is the complex of subunit proteins that may be part of a larger supercomplex of a photosystem and is the functional unit in photosynthesis, devoted to the absorption of sunlight. The energy excitation is first tunnelled among other surrounding molecules of the same complex and then from one LHC to another and then funnelled to reaction centres (RCs), where it is converted into charge separation with 90% quantum efficiency.

The presence of proteins in LHC complexes is attributable to the fact that Chl of RCs cannot absorb sunlight at an efficient rate that is enough for efficient photosynthesis to occur. In fact, Chl molecules in RCs absorb only a few photons each second, which are insufficient to drive electron transport into chloroplast membranes (present in 1 RC of about 300 antenna molecules). To overcome this problem, RCs are associated with antenna pigment-protein complexes that absorb sunlight and very efficiently transfer it to RCs. For the importance of the LHCs in gathering sunlight, they differ in the number of pigments and in their composition and structure in a way that they are an optimized energy collector system (**Figure 3**). The proteins play an important function in the precise position, mutual separation and relative orientation of antenna.

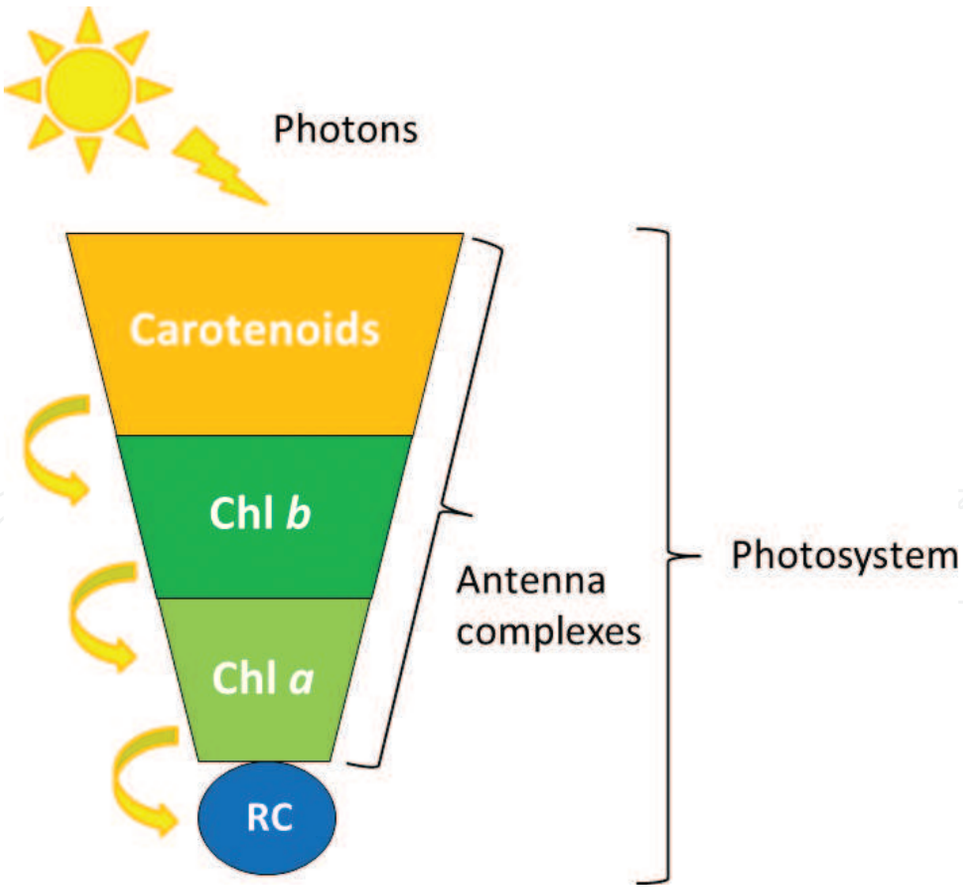

Figure 3. A schematic representation of the light absorption process of chloroplasts. Antenna complexes, composed of carotenoids, Chl *a* and Chl *b* molecules, absorb photons from sunlight and transfer them to the RC, which consists of a special couple of molecules of Chl *a*. Antenna complexes and the RC form a photosystem.

Photosynthetic unit (PSU) represents the basic unit of the light-harvesting apparatus and consists of a large number of antenna chromophores coupled to a RC. Excitation-transfer pathways follow a scheme in which different chromophores build an energy funnel where chromophores, which absorb in the blue side of spectrum, transfer excitation energy to more red-shifted chromophores (**Figure 3**). Theoretically, the PSUs are considered individual entities but [2] proposed the *lake* and *puddle* model. In the second model, the PSUs do not interact with each other and the excitation light absorbed by chromophores is always transferred to the same RC. Differently, in the *lake* model, the antenna chromophores form a matrix with embedded RCs in which there is an unrestricted energy transfer.

2. Charge separation in photosystems and electron transport

Photosynthesis starts with light absorption by the chromophores, which excites the molecules from the ground state to an electronic excited state. Once sunlight energy is absorbed, pigments in the excited state have a short life and relax to the ground state after about 4 ns [3]. The singlet excited state lifetime of Chl is lower compared with the radiative lifetime, largely owing to intersystem crossing, which yields triplet excited states of Chl (about 10 ns) [4]. This electronic excitation must be usefully harvested before the molecules relax, and this happens when excitons are transferred through space among chromophores until they reach, eventually, a RC where charge separation occurs. In plants, there are two RCs constituted by two Chl molecules, P680 and P700, respectively, for PSII and PSI, and Chl with absorbance maxima corresponding to these wavelengths is proposed as the final slight sink. These chlorophylls drive electron transfer by charge separation, a reaction in which P680 and P700 molecules reduce an acceptor. These driving reactions energetically downhill from the potential that is more negative to ones that are more positive (**Figure 4**). All these electron transfer steps in photosynthesis share a common feature. The loss of an electron from one component, which remains in an oxidized state, reduces another one. Typically, electron transport carriers are small molecules or atoms of metallic elements that can exist in a number of valence states.

In photosystem RCs, the light-induced loss of an electron (charge separation) leaves P680 and P700 in an oxidized state ($P680^+$ and $P700^+$) and the respective acceptors, pheophytin for P680 and A_0 (chlorophyll), in a reduced state. $P680^+$ is reduced from an adjacent tyrosine molecule (TyrZ) in the polypeptide chain of the D1 protein of the PSII complex. In turn, the oxidized is reduced by electrons from the oxygen-evolution complex (OEC) that oxidized water. Two water molecules are oxidized to produce oxygen, four protons and four electrons that are transferred one at a time. These redox reactions are carried out by OEC that consists of four manganese atoms held in a protein matrix with one atom of calcium and chlorine each (**Figure 4**). This process is known as a S-cycle from [5] that provides protons derived from water oxidation to be released into the lumen of the thylakoid membranes.

In the other set of reactions, reduced pheophytin is oxidized by passing an electron to the first of two plastoquinone (PQ) molecules, tightly bound at the site Q_A of D2 protein in the PSII. Then, via an iron atom, an electron is transferred to the next PQ at the site Q_B. Both PQs require two electrons for their complete reduction; at the Q_A site, PQ undergoes to a single

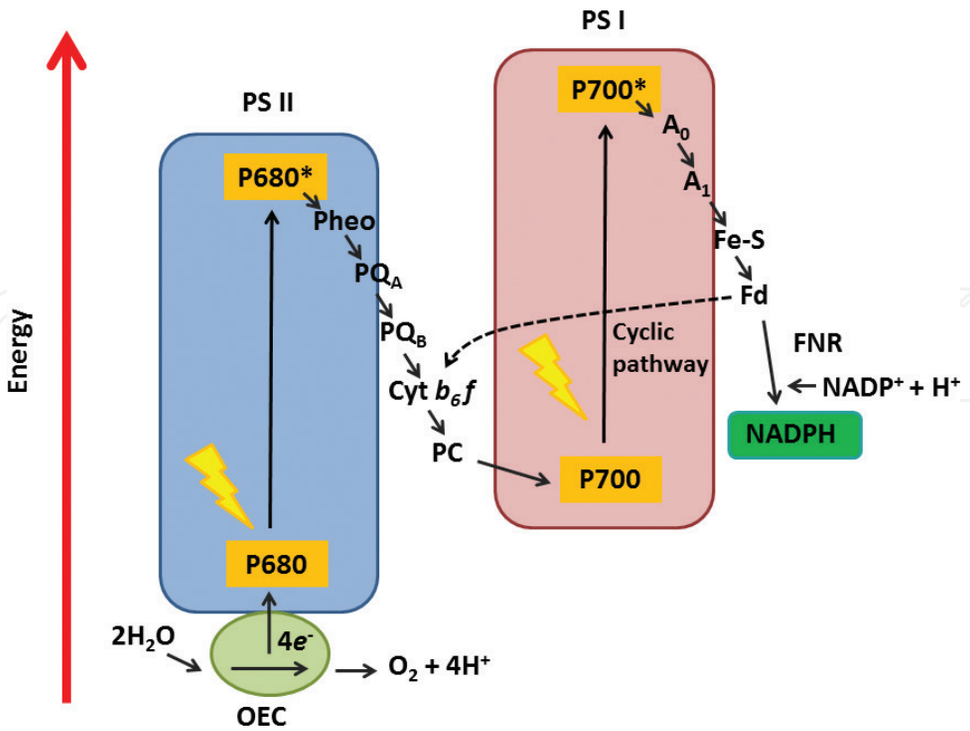

Figure 4. A representation of the linear non-cyclic (solid line) and cyclic electron flow (dashed line) in the chloroplast membranes. OEC tetranuclear Mn cluster; P680, reaction centre of photosystem II (PSII); P680*, excited electronic state of P680; Ph, pheophytin; Q_A and Q_B, plastoquinone; protein complex containing cytochrome b_6 and cytochrome *f*; PC, plastocyanin; P700, reaction centre of PSI; P700*, excited electronic state of P700; A_0, a special chlorophyll *a* molecule; A_1, phylloquinone; Fe-S, iron sulphur centres; Fd, ferredoxin; NADP, nicotinamide-adenine dinucleotide phosphate and FNR, ferredoxin-$NADP^+$ reductase.

reduction event to the semiquinone state before being re-oxidized by the PQ at Q_B site. Two successive reductions occur that fully reduce PQ at Q_B site, which, for its reduction, requires also two protons from the stromal side of the membranes and forms PQH_2 that leaves PSII and diffuses in the lipid bilayer, representing a mobile carrier of protons and electrons. A new molecule of PQ (in oxidized form) replaces this plastoquinone in the Q_B site.

PQH_2 formed by the PSI activity represents the substrate of the Q cycle on cytochrome b_6f, another integral transmembrane protein complex on thylakoid membranes. PQH_2 is oxidized in two steps to PQ. The first step happens at Q_p site, located on the luminal side of cytochrome b_6f, and the electron is transferred at the end to plastocyanin (PC), a soluble small protein containing copper. The second electron is transferred until Q_n site located on the stromal side of the cytochrome where it reduces further PQ molecule to semiplastoquinone. Another PQH_2 molecule originating from PSII is oxidized in the same two steps at the Q_p site, generating further a reduced plastocyanin and completing the reduction of semiplastoquinone to PQH_2. The oxidation of PQH_2 at Q_p site determines the release of two protons in the lumen that represents the most important feature of the Q cycle. In fact, this cycle acts as a proton pump, essential to generate the transmembrane electrochemical H^+ gradient.

After light absorption and charge separation in PSI, $P700^+$ is generated, and it is reduced back to P700 by direct interaction with reduced PC diffusing from cytochrome b_6f complex.

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Plastocyanin, from its copper atom, reduces directly $P700^+$. The electron flow generated by charge separation that occurs in P700 determines the reduction of different carriers, and the final electron acceptor is represented by Fd, a small water-soluble iron-sulphur protein. Reduced Fd is capable of reducing a variety of molecules. Usually, it reduces $NADP^+$, which requires two electrons and two protons to yield NADPH in a reaction catalyzed by ferredoxin-$NADP^+$ reductase (FNR) (**Figure 4**), thus completing the so-called Z scheme. The electron flow generates even chemical energy, that is ATP, by the enzymatic activity of ATP-ase, a transmembrane complex that utilizes the proton gradient generated by Q cycle and water oxidation, to synthetize ATP.

3. Excess of excitation energy

In the past, the higher order structure of PSII was thought to be important only to increase the efficiency of light harvesting; nowadays, it has been suggested that it provides the essential dynamic properties involved in its regulation [6]. When light is low, in a way, extremely efficient antenna systems absorb light and tunnel it through RC, but when light is in excess, a large extent of this energy is dissipated, overall as heat, to prevent photo-damage to PSUs. When plants are exposed to shade or sunlight conditions, different mechanisms occur. Shade leaves are typically larger in area but thinner than sun leaves because they develop shorter palisade cells. In shade leaves, the chloroplasts move within the cells to take up a position where they will absorb the maximum light without shading other chloroplasts below. In addition, shade leaves show a large number of antenna, and usually, the peripheral antenna are rich in Chl *b* molecules (Chl *a*/*b* = 1.33). All these mechanisms enhance and optimize the light absorption. However, even shade leaves have adapted mechanisms aimed to regulate the light absorption, as the state II-I transition (also called spillover process). The aim of this process is the reduction of light tunnelled to P680 altering the ratio of light energy absorbed between PSII and PSI. In fact, RCs of the two photosystems have different absorption spectra (high energy is absorbed by P680 as compared with P700), and this determines that when the energy flow through each is not balanced to the requirement of the Z scheme, an excess of energy could accumulate in the system. In this way, LHCII trimers represent a feedback loop that adjusts the amount of antenna Chls, providing energy to each photosystem (state transition). The excess of light energy flowing through PSII RCs is higher than that flowing through PSI RCs, conditions in which an excess of reduced PQ occurs. This activates a kinase that phosphorylates some LHCII trimers, and this extra charge allows them to dissociate from the PSII (state II) and migrate towards the stroma lamellae (state I transition) where they bind to the PSI complex, increasing in this way the flow through the system. The increase of PSI activity leads to the oxidation of reduced PQ, which activates a phosphatase that removes the phosphate group to the LHCII trimers that return to PSII (state II transition).

In contrast, sun leaves live in very high radiation levels overall at the top of the canopy. The light response curve in relation to the light intensity shows that the amount of energy utilized is lower than that absorbed because the light energy utilized in carbon reduction is mostly due to the limitation on the rate of CO_2 diffusing into the leaf (**Figure 5**). In these conditions,

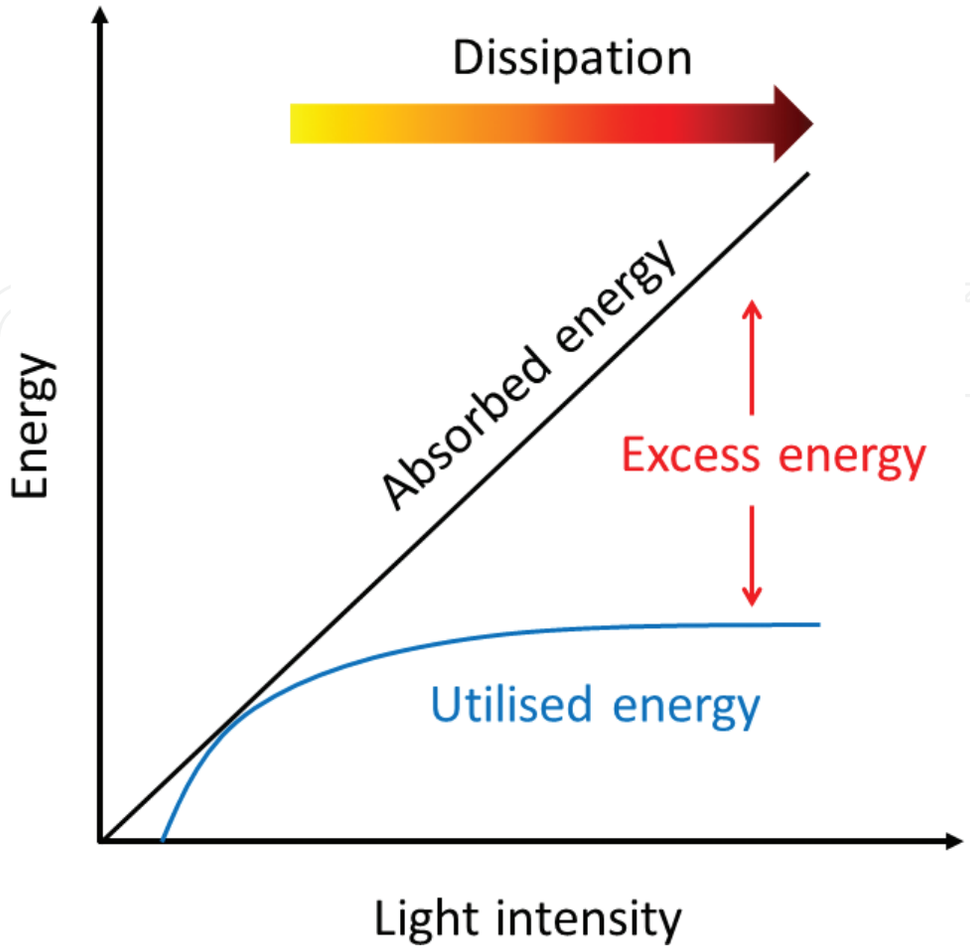

Figure 5. Absorbed and utilized energy in response to increasing light intensities. When light absorbed exceed photosystems requirement, the 'excess energy' can potentially cause photo-oxidative damage if it is not efficiently dissipated.

the antenna Chls become saturated and tunnel a high flow of the excitation energy to the RC that cannot be dissipated along the electron flow. The excess of energy must be efficiently dissipated through different mechanisms in order to avoid photo-damage to PSII.

Photosystem II is particularly sensitive to photoinhibition because the high redox potential of the oxidized P680 ($P680^+$), on the other hand, necessary for water oxidation. Accumulation of $P680^+$ leads to different types of photoinhibition:

(i) Acceptor-side photoinhibition: when reduced PQ is not re-oxidized, the $P680^*$ charge recombination is inhibited and P680 is expected to lead to the triplet state of P680, $^TP680^*$. This chemical species may react with oxygen and produce harmful singlet oxygen.

(ii) Donor-side photoinhibition: if the OEC is chemically inactivated, the donation of electrons from water does not keep up with the electron transfer from P680 to the acceptor side. In this case, an accumulation of $P680^+$ occurs. The high redox potential of this chemical species induces the oxidation of various organic components such as proteins or pigments until damage is done to D1 protein of PSII.

Different mechanisms are present in PSII aimed to dissipate the excess of photons absorbed by antenna, and different defence lines occur into the chloroplast.

4. First line of defence of chloroplast: dissipation of excess excitation light

First line of chloroplast defence includes suppression mechanisms aimed to reduce or dissipate the excitation light tunnelled in P680. At leaf level, the change in the leaf angle with respect to the incident light and/or the chloroplast movement into the leaf to self-shading positions along the sidewalls of cells represent mechanisms by which a decrease in absorbed light can occur.

In the chloroplast, there are essentially three mechanisms to contrast the high light conditions: adjustment in synthesis and amount of antenna protein, movement of LHCII (state II-I transition) and non-photochemical quenching [7]. The first of these mechanisms is related to the expression of *Lhcb* genes, whose expression is downregulated by high light conditions and/or low CO_2 concentration. The sensor mechanism is not known even though one possible candidate is the redox potential (i.e., the level of reduced PQ) [8], but also ROS represent possible signal molecules [9, 10]. Clearly, these slow mechanisms cannot entirely prevent the accumulation of excess of energy in the antenna system. However, photosynthesis in green plants depends on protective mechanisms that adapt within minutes or seconds to changing light conditions. Excited Chls return to the ground state either by emitting photons (fluorescence) or by dissipating it as heat. All these mechanisms aimed to remove this trapped energy before it passed on down the electron transport chain are named *non-photochemical quenching* (NPQ). NPQ is heterogeneous and composed by at least three components: the major and rapid component is the pH- or energy-dependent component qE, a second component qT, related to the phenomenon of state transition but negligible in most of plants under excess light and the third and slow component, qI, related to the photoinhibition of photosynthesis [11].

It has been reported that two distinct qE mechanisms occur, one involving zeaxanthin (Zea) (quenching type 1) and the other carotenoid lutein (Lut) (quenching type 2) [12]. In qE type I, three xanthophylls, violaxanthin (Vio), anteraxanthin (Ant) and Zea, are involved in the well-known xanthophyll cycle in which the epoxidation of Vio to Zea via Ant determines an efficient dissipation of excess light into heat [13]. Electron flow pumping and generating protons in the lumen decrease its pH from about 7 to less than 5; this represents a strong signal that starts a series of quenching processes. The low pH-induced protonation of PsbS peptide, for its proximity to antenna complexes (CP24, CP26 and CP29), induces in turn in these complexes conformational changes. In the chemical state, antenna complexes bind one molecule of Zea and one of Chl (*Zea-Chl complex = quenching complex*) that accept energy transfer from excited Chls. Zeaxanthins are able to return to their ground state dissipating energy as heat :

$$\text{LHCII}^{*} + \text{zeaxanthin} \rightarrow \text{LHCII} + \text{zeaxanthin}^{*}. \quad (1)$$

$$\text{Zeaxanthin}^{*} \rightarrow \text{zeaxanthin} + \text{heat}. \quad (2)$$

It has been reported that in the crystal structure of LHCII is present Vio, and its peripheral localization suggests that it could be de-epoxidized to Zea by Vio de-epoxidase (VDE), an enzyme that is activated by low lumen pH occurring in high light conditions. The back reaction by Zea epoxidase is slow and causes a sustained quenching that relaxes within 1–3 hours following light stress and depends on the release of Zea from antenna pigments. In conclusion, Zea is certainly considered a regulator of light harvesting for its role in the xanthophyll cycle and carries out three fundamental roles during high light conditions: (i) protection against photo-oxidation due to radical oxygen's attack (because it quenches oxygen singlet energy), (ii) absorption of Chl triplet energy and (iii) absorption of incoming photons and transferring them to neighbouring Chl molecules increasing in this way the overall absorption spectrum of the PSs [14]. In addition, it has been reported that this xanthophyll exhibits an antioxidant function in the thylakoid membrane [15].

In addition, trimeric LHCII binds other types of xanthophylls: two all-*trans*-luteins and a 9-*cis*-noexanthin [16]. The minor monomeric complexes CP24, CP26 and CP29 all bind Lut, and in addition, CP29 binds two xanthophyll cycle carotenoids and one-half to one neoxanthin (Neo), CP24 binds two xanthophyll cycle carotenoids and CP26 binds one xanthophyll cycle carotenoids and one Neo [17, 18]. In the quenching type 2, qE is an intrinsic LCHII property: protein conformational changes alter configurations of bound pigment (normally Lut), which become an efficient quencher of Chl-excited state [12]. A change in the conformational state of another LHCII-bound xanthophyll, Neo, correlates with the extent of quenching. In the model for type 2 quenching proposed by [19], Zea acts not as a quencher but as an allosteric modulator of the ΔpH sensitivity of this intrinsic LHCII quenching process. The two types of quenching involved different xanthophylls that operate at different sites, but there are some similarities in the reasons that both involve ΔpH and PsbS-mediated conformational changes [12].

Given that the xanthophyll cycle quenches only 95% of the triplet Chl [20], the unquenched triplet Chl is the reason for the need of singlet oxygen not only scavenging by carotenoids bound to LHCII but also by carotenoids free in lipid matrix [21]. Lut has the specific property of quenching harmful 3Chl* by binding at site L1 of the major LHCII complex and of other Lhc proteins of plants, thus preventing ROS formation [20]. Neo contributes PSII photoprotection in a dual way: determins conformational change in trimeric LHCII, which reduces light absorption and controls the accessibility of the O_2 to the inner core of the complex [20, 22]. The trimeric organization of LHCII is, definitively, effective in screening the internal protein domain from molecular oxygen [23].

5. Second line of defence of chloroplast: antioxidant enzymes and molecules

As reported above, the excess of excitation energy induces an excess of singlet-excited Chl *a* that is de-excited via thermal dissipation. However, the remaining singlet-excited Chl *a* can convert to triplet-excited Chl that readily reduces molecular oxygen. This determines the synthesis of ROS that is potentially dangerous to organic molecules in the chloroplast. In the second line of defence, antioxidant molecules and enzymes that together scavenge ROS play a key role.

The primary products of molecular oxygen reduction are disproportionate to H_2O_2 and O_2 in a reaction catalyzed by superoxide dismutase (SOD). H_2O_2 produced is then reduced to water with the reducing power of ascorbate (ASA) in a reaction catalyzed by ASA peroxidase (APX), and ASA is oxidized to monodehydroascorbate (MDHA) that is directly reduced to ASA by reduced ferredoxin or NADPH by MDHA reductase. Alternatively, MDHA is spontaneously disproportionated to dehydroascorbate (DHA) and ASA. DHA is then reduced by reduced glutathione (GSH), by the enzyme DHA reductase that produces oxidized glutathione (GSSG) and ASA. Finally, GSSG is reduced again in GSH by the action of GSH reductase, and the reducing power is represented by reduced Fd or NADPH, that, in turn, are reduced by PSI activity. This indicates that any pathway aimed to regenerate ASA utilizes electrons derived from water. For this reason, the previous process is referred as water-water cycle [10].

In addition to the primary antioxidant systems, carotenoids have a protective role against ROS since they are very efficient physical and chemical quenchers of singlet oxygen and potent scavengers of other free radicals [24]. For example, β-carotene, located in the core complex of both PSII and PSI, plays a role as a quencher of Chl triplet and singlet oxygen [25], and the products generated from the oxidation of β-carotene by singlet oxygen represent primary sensor signalling under oxidative stress [26]. Other carotenoids play an important role as antioxidants in the chloroplast. Lut is the most abundant carotenoid in the chloroplast and is required as a quencher [7], while Neo can scavenge superoxide anion [27]. The antioxidant activity of carotenoids is carried out in combination with other lipophilic antioxidants. In this way, it has been reported that Zea, in cooperation with tocopherol, prevented photo-oxidation induced by high light [28], or a strong increase in carotenoids pigment (including those involved in xanthophyll cycle) is reported together with the activity of SOD enzyme following oxidative stress [29]. Again, carotenoids can influence the structure and fluidity of thylakoid membranes [30], that is essential for photosynthetic functions, influence barrier status to ions and oxygen, increase thermostability and protect against lipid peroxidation. In fact, as reported by [30], β-carotene can fluidize the membrane because it can move in the inner hydrophobic part of the membrane, and xanthophyll (and in particular Zea) shows the polar group that orientates these carotenoids perpendicular to the membrane surface.

6. From PSII repair processes to alternative electron sinks

In the last 30–40 years, the susceptibility of D1 protein to photo-damage has been well known, and the concept of the replacement of the damaged D1 protein during the repair cycle of PSII is extensively investigated [13, 31–33]. Moreover, D1 damage has been shown to be directly proportional to light intensity [34].

The repair process of photo-damaged D1 proteins consists of different steps: (i) prompt, partial disassembly of the PSII holocomplex, (ii) exposure of the photo-damaged PSII core to the stroma of the chloroplast, (iii) degradation of photo-damaged D1, (iv) *de novo* D1 biosynthesis and insertion in the thylakoid membrane and (v) re-assembly of the PSII holocomplex, followed by activation of the electron-transport process through the reconstituted D1/D2 heterodimer [35]. The sequence leading to the recovery of photo-damaged PSII is consistent with

the frequent D1 turnover in the chloroplast and with the heterogeneity in the configuration and function of PSII.

In the past, the sensibility of PSII was linked to an inherent defect of photosynthetic apparatus but now it is clear how this mechanism of damage-repair of PSII is extremely regulated [33] and protects even PSI from irreversible damage. In fact, the repair mechanisms in PSI are time and high energy consuming, and it has been suggested that the inhibition of PSII is likely to protect PSI [33].

Reduced Fd plays an important role in preventing the over-reduction of electron flow, and a wide range of electron sinks are available in chloroplasts. Electrons are preferentially utilized by the FNR enzyme that produces NADPH for CO_2 photoassimilation or ferredoxin:thioredoxin reductase that synthesizes thioredoxin responsible for the regulation of some enzymes of Calvin-Benson cycle [36]. On the other hand, reduced Fd can release electrons also to ferredoxin:nitrite reductase and sulphite reductase for the reductive assimilation of nitrite [37] and sulphur [38]. Finally, reduced Fd represents an electron donor for fatty acid desaturases [39] and glutamine:oxoglutarate amino transferase [40]. However, when $NADP^+$ is not available, reduced Fd releases its electron to different acceptors whose function is to avoid an over-reduction of PSI [41]. It has been discovered that there is an electron transport driven solely by PSI and scientists called it cyclic electron flow. In this cycle, electrons can be recycled from reduced Fd to PQ and subsequently, to the cytochrome b_6f complex via the Q cycle [42]. Such cyclic flow generates ΔpH and thus ATP without the accumulation of reduced species. In addition, the generated ΔpH may regulate photosynthesis via NPQ (see Section 4). Another electron acceptor of reduced Fd is molecular oxygen inducing the pseudo-cyclic electron flow. The reduction of molecular oxygen with one electron generates superoxide anions in the so-called Mehler reaction, which restores the redox poise when linear electron flow is over-reduced [43]. The radical oxygen species is efficiently removed by water-water cycle. Chlororespiration is another effective electron sink in which reduced Fd is directly involved. In this process, two enzymes play the key role: NADH dehydrogenase complex and nucleus-encoded plastid-localized terminal oxidase (PTOX). The enzyme PTOX catalyzes the reaction in which electrons are transferred from PQH_2 to molecular oxygen forming water [44].

Finally, in addition to the above-reported electron flow, photorespiration is another efficient pathway by which plants adjust the ATP/NADPH ratio and consume the excess of excitation energy.

7. Conclusions

Certainly, Chls represent the key molecules involved in light energy absorption and transduction into chemical energy. Chls absorb the light energy that reaches leaves in a very efficient manner but sometimes, light exceeds photochemistry requirement, and the complexity of photosystems is essential to modulate and dissipate excess of excitation energy. A wide range of responses to environmental stimuli thus characterizes the photoprotection of chloroplasts. The increasing level of complexity from the molecular (pigments and protein) to supramolecular

(photosystems) level mirrors the necessity of different time-scale responses (from seconds to months) to modulate light that is (inevitably) absorbed. In the range of seconds to minutes, modulation of the redox state of photosynthetic electron transport activates the non-photochemical quenching of excess of excitation energy not only through xanthophyll cycles [13] but also by II-I state transition [45]. On a larger scale (minutes to hours), modulation of redox state of electron transport induces changes in gene expression (organellar and nucleus) through retrograde regulation that changes the structure of the photosynthetic apparatus [46, 47]. On the time scale from weeks to months, the redox state of electron transport determines changes in plant growth and morphology [48].

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