Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo?

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Organisms that rely on oxygenic photosynthesis are subject to the effects of photo-oxidative damage, which impairs the function of photosystem-II (PSII). This phenomenon has the potential to lower rates of photosynthesis and diminish plant growth. Experimental evidence shows that the steady-state oxidation-reduction level of the primary quinone acceptor (Q$_A$) of PSII is the parameter that controls photodamage under a variety of physiological and environmental conditions. When Q$_A$ is oxidized, excitation energy at PSII is dissipated via a charge-recombination reaction. Such non-assimilatory dissipation of excitation generates singlet oxygen that might act to covalently modify the photochemical reaction center chlorophyll. Under steady-state photosynthesis conditions, the reduction state of Q$_A$ increases linearly with irradiance, thereby causing a correspondingly linear increase in the probability of photodamage. It is concluded that there is a low probability that photodamage will occur when Q$_A$ is oxidized and excitation energy is utilized in electron transport, and a significantly higher probability when Q$_A$ is reduced in the course of steady-state photosynthesis.

Life on earth is sustained by oxygenic photosynthesis, a process that begins with the utilization of sunlight for the oxidation of water molecules. The chemical energy stored in this endergonic oxidation is processed through the electron-transport chain of the chloroplast thylakoids and, eventually, is delivered in the form of reductant (reduced ferredoxin) and high-energy phosphate bonds (ATP). The absorption of light and the conversion of excitation energy to chemical energy takes place in photosystem-II (PSII) and photosystem-I (PSI) in the thylakoid membrane. Light energy in PSII specifically facilitates the generation of a strong oxidant that is capable of oxidizing water molecules. The ability of PSII to extract electrons and protons from water was undoubtedly a significant event in the evolution of life on earth. By contributing to the gradual accumulation of oxygen in the atmosphere, it has enabled the evolution of oxidative phosphorylation. For this reason PSII is known as ‘the engine of life on earth’.

From a biochemical point of view, PSII is a specialized water-to-plastoquinone oxidoreductase. This specialized enzyme features a sizable holocomplex, consisting of 25–35 transmembrane and peripheral proteins. Many of the transmembrane proteins function as chlorophyll–protein light-harvesting complexes. The functional center of this holocomplex contains the so-called D1/D2 32/34 kDa heterodimer proteins, which perform the light utilization, water oxidation and electron transfer reactions to plastoquinone. From this biochemical point of view, PSII is a specialized water-to-plastoquinone oxidoreductase. This specialized enzyme features a sizable holocomplex, consisting of 25–35 transmembrane and peripheral proteins. Many of the transmembrane proteins function as chlorophyll–protein light-harvesting complexes. The functional center of this holocomplex contains the so-called D1/D2 32/34 kDa heterodimer proteins, which perform the light utilization, water oxidation and electron transfer reactions to plastoquinone.

These highly specialized functions of PSII take place in an oxygen-saturated microenvironment, where photons, in the form of excitation energy, arrive at a rate of up to 10 000 per s. The transient formation of strong oxidants, the abundance of oxygen and the arrival of excitation energy at high rates can lead to photo-oxidative damage. Indeed, such photodamage occurs frequently within the reaction center of PSII. It causes an irreversible inhibition in the function of the reaction center chlorophyll (P680) in the D1 protein and stops photosynthesis.

Through the process of two billion to three billion years of evolution, organisms of oxygenic photosynthesis have not evolved systems to prevent photodamage from occurring. Thus, every oxygen-evolving photosynthetic organism known, from cyanobacteria to C4 plants, is subject to irreversible photodamage. However, photosynthesis has evolved a highly specialized repair mechanism that restores the functional status of PSII. This PSII damage and repair cycle is important for the function and productivity of photosynthesis. It has been estimated that, in the absence of the repair mechanism, photodamage would lower the yield of photosynthesis to <5% of the yield achieved now. Life on earth could not have evolved to present-day levels in the absence of this PSII repair process.

![Fig. 1. Temporal sequence of events in photosystem-II (PSII) damage, holocomplex disassembly, and degradation and replacement of the D1/32 kDa reaction center protein. The rate constant of photodamage (k$_{photodamage}$) depends on the incident light intensity 19–21. The rate of PSII disassembly (k$_{diss}$) and D1 degradation (k$_{degrad}$) become rate limiting under high light intensities. The rate of D1 degradation is the overall rate-limiting step of the PSI repair process. It was estimated to occur with a half time of 60 ± 15 min (Ref. 9). When the rate of photodamage is faster than the rate of repair, photodamaged PSII complexes accumulate in the thylakoids 75. This condition is known as photoinhibition of photosynthesis. It causes significant losses in plant growth and productivity.](image-url)
In broad terms, photodamage to D1 is followed by:

• Prompt, partial disassembly of the PSII holocomplex.
• Exposure of the damaged PSII core to the stroma of the chloroplast.
• Degradation of photodamaged D1.
• De novo D1 biosynthesis and insertion in the thylakoid membrane.
• Re-assembly of the PSII holocomplex, followed by activation of the electron-transport process through the reconstituted D1/D2 heterodimer.

There is a dynamic relationship between photodamage and repair (Fig. 1). The interplay between these two processes will define whether there is an adverse effect on photosynthesis. For example, when the chloroplast repair process cannot keep up with the rate of photodamage, the productivity of the photosynthetic apparatus declines and plant growth diminishes. This condition is known as photoinhibition of photosynthesis; it occurs whenever the rate of electron-transport process through the reconstituted D1/D2 heterodimer is altered in a way that might lead to photo-oxidative damage. In a temporal sequence of events, excitation energy in P680 (denoted by *) leads to a primary charge separation between P680 and pheophytin (reaction half time of 3 ps), followed by electron-transfer from Ph to Q\(_a\) (half time of 200 ps), and from Q\(_a\) to Q\(_b\) (half time of 400–800 µs). On the donor side of PSII, the positive charge on P680* is neutralized by electron-transfer from Y\(_z\) (reaction half time of 20–200 ns). Electron donation from the tetranuclear Mn complex to Y\(_z\)* (not shown) serves both to store the oxidizing equivalent and to neutralize the primary (P680) and secondary (Y\(_z\)) electron-donor molecules. The stepwise accumulation of four positive charges on the tetranuclear Mn complex in PSII constitutes a necessary and sufficient condition for the oxidation of two H\(_2\)O molecules, the release of four electrons, four protons, and of molecular O\(_2\). For the purposes of this paper, these can be divided into steady-state electron-transfer reactions when the primary quinone acceptor Q\(_a\) is reduced.

Electron-transfer reactions when Q\(_a\) is reduced

When Q\(_a\) is reduced at the rate of a primary charge separation between P680 and Ph, the sequence of electron-transfer reactions is altered in a way that might lead to photo-oxidative damage. For example, when Q\(_a\) is reduced, the P680* Ph Q\(_a\) Q\(_b\) 1 \(\rightarrow\) P680 1 O\(_2\) (Eqn 4) reaction is altered in a way that might lead to photo-oxidative damage. In such a photo-synthesis versus light intensity curve (Fig. 2), the rate of photo-synthesis first increases linearly with light intensity and then levels off as saturating light intensity (I\(_s\)) is approached. The slope of the initial linear increase provides a measure of the photon yield of photosynthesis (O\(_2\) produced per photon absorbed). The rate of photosynthesis reaches saturation at light intensities > I\(_s\), the light-saturated rate (P\(_{sat}\)) provides a measure of the capacity of photosynthesis for the leaf or algal sample. It is evident that light absorption and utilization by the photosynthetic apparatus is altered in a way that might lead to photo-oxidative damage. For example, when Q\(_a\) is reduced, the P680* Ph Q\(_a\) Q\(_b\) 1 \(\rightarrow\) P680 1 O\(_2\) (Eqn 4) reaction is altered in a way that might lead to photo-oxidative damage. In such a photo-synthesis versus light intensity curve (Fig. 2), the rate of photo-synthesis first increases linearly with light intensity and then levels off as saturating light intensity (I\(_s\)) is approached. The slope of the initial linear increase provides a measure of the photon yield of photosynthesis (O\(_2\) produced per photon absorbed). The rate of photosynthesis reaches saturation at light intensities > I\(_s\), the light-saturated rate (P\(_{sat}\)) provides a measure of the capacity of photosynthesis for the leaf or algal sample. It is evident that light absorption and utilization by the photosynthetic apparatus is altered in a way that might lead to photo-oxidative damage.
algae and cyanobacteria will encounter an imbalance between the processes of light absorption and utilization, the magnitude of which will depend on light intensity and on the photosynthesis saturation intensity \(I_s\). For example, in higher plants and green algae, the rate of photodamage is modulated by the PSII light-harvesting apparatus, which will absorb photons that cannot be efficiently utilized in the process of oxygen production or \(CO_2\) fixation. The excess photon absorption processes will be dissipated by non-assimilatory photochemistry, the extent of which is expected to increase linearly with light intensity beyond the \(I_s\) level.

**Dependence of photodamage on light intensity**

On the basis of the mechanism for \(D1\) photodamage, it has been assumed that photodamage will be accentuated when there is imbalance between light energy absorption and utilization at PSII. According to this hypothesis, photodamage should be minimal at light intensities \(\sim I_s\) and become significant at light intensities \(\sim I_s^*\). Thus, the rate of photodamage was expected to be a non-linear function of light intensity. However, this notion was questioned in recent studies that addressed the dependence of photodamage on light intensity.

The rate constant for photodamage was shown to be a linear function of light intensity in the physiological range of light intensities (Fig. 3), both in a higher plant (pumpkin) and in a green alga (*Dunaliella salina*). The linear dependence of the rate constant for photodamage on irradiance suggests a simple probability for photodamage every time excitation energy arrives at the PSII reaction center, irrespective of the rate of photosynthesis, that is, irrespective of the photochemical utilization or non-assimilatory dissipation of the absorbed photons. According to this straightforward model, as the light intensity increases, so does the rate of light absorption and excitation energy transfer to a reaction center, thereby increasing the rate constant for photodamage. This interpretation is consistent with evidence showing a reciprocity of irradiance and duration of photoinhibition, indicating that PSII photodamage depends on the total number of photons absorbed and not on the rate of photon absorption per se. Such experimental results gave rise to the notion that PSII might be a ‘photon counter’, implying that photodamage occurs after a fixed number of photons have been absorbed by PSII, irrespective of the electron transport status of the photosynthetic apparatus, putting the mechanism of \(D1\) photodamage into question.

**Photosystem-II chlorophyll antenna size modulates the rate of photodamage**

If PSII photodamage depends strictly on light absorption by the chloroplast, then it follows that the size of the PSII light-harvesting chlorophyll (Chl) antenna must modulate the rate of this adverse phenomenon directly. Whether the light-harvesting Chl antenna size of PSII affects the rate of photodamage is controversial. Earlier work with isolated thylakoid membranes from wild type and chlorina F2 mutant barley indicated that the rate of photodamage is independent of the PSII antenna size. Under identical incident light intensities, the Chl \(b\)-less chlorina F2 mutant sustained slower rates of photodamage than the corresponding wild type 22,23. It has also been reported that photoinhibition is totally independent of the size of PSII chlorophyll (Chl) antenna in green algae 24,25. Re-examination of this question 26 supports the notion that the rate of photodamage is modulated by the PSII Chl antenna size (Fig. 4) and is consistent with the notion of a PSII ‘photon counter’.

**Electron transport and photosynthesis mitigate against photodamage**

Until recently, the role of PSI electron transport in the mitigation against photodamage was also unclear. Earlier studies suggested that a limitation in the rate of electron flow, caused by low CO\(_2\) partial pressures, might attenuate photoinhibition in cyanobacteria 27 and higher plants 28. It has also been reported that electron transport to oxygen via the photosynthetic oxidase 29 or the Mehler reaction 30 can protect against photoinhibition in pea leaves (but see Ref. 34). However, antisense transgenic plants with a substantially lower cytochrome \(b/f\)-complex content, in which illumination produced slow rates of linear electron transport and in which \(Q_a\) accumulation in the reduced state, did not show the expected increase in their susceptibility to photoinhibition 31. One possible reason for the confusion generated from these apparently contradictory results is that frequently photoinhibition is measured rather than the rate of photodamage. Photoinhibition is a function of both photodamage and repair and, therefore, measurements of photoinhibition are always more difficult to interpret. A more thorough study in this direction was undertaken with *Dunaliella salina* 28. Cells were grown under high irradiance, either with a limiting supply of inorganic carbon, provided by addition of 25 mM NaHCO\(_3\), to the medium \([P_{\text{CO}_2}] = 100 \text{ pmol} O_2 (10^6 \text{ cells})^{-1} \text{s}^{-1}\), or with 5% CO\(_2\) in air, bubbled into the culture \([P_{\text{CO}_2}] = 250 \text{ pmol} O_2 (10^6 \text{ cells})^{-1} \text{s}^{-1}\). These conditions differed by a factor of \(c.2\) in the rate constant of photodamage (Fig. 4), supporting the concept that photochemical utilization of excitation energy in the electron-transport process mitigates against photodamage. These results are not consistent with the notion of a PSII ‘photon counter’.

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**Fig. 2.** Light-saturation curve of photosynthesis (\(P\) versus \(I\)) in the green alga *Dunaliella salina*. Rates of oxygen production were measured on a per chlorophyll (Chl) basis. Note the linear increase in the rate of photosynthesis at low intensities and photosynthesis saturation approached at \(I_s\) of \(\sim 250 \mu\text{mol photons m}^{-2} \text{s}^{-1}\). The light-saturated rate \(P_{\text{max}}\) of this sample was \(\sim 100 \text{ pmol} O_2 (\text{mol Chl})^{-1} \text{ s}^{-1}\).
Suboptimal temperature accentuates photodamage and photoinhibition

Exposure of plants to chilling temperatures lowers the irradiance threshold for the manifestation of photoinhibition, partly because chilling temperatures, acting in a species-dependent manner, slow down the repair of the photosynthetic apparatus. Suboptimal temperatures also enhance the rate of photodamage as they slow down electron transport and shift the steady-state redox level of QA, resulting in an increased proportion of closed PSII reaction centers. Conversely, there is a significantly higher probability for photodamage when QA remains reduced during illumination, such as when forward electron flow is slowed down or blocked and excitation energy dissipates via charge-recombination reactions in a non-assimilatory process.

Such a hypothesis on the regulation of photodamage by the redox state of QA requires that a linear increase of the rate constant of photodamage (Fig. 3) must then underline a linear increase in the inherent probability for photodamage when QA is oxidized and excitation energy dissipates by useful photochemistry in the form of linear electron transport through PSI. Conversely, there is a significantly higher probability for photodamage when QA remains reduced during illumination, such as when forward electron flow is slowed down or blocked and excitation energy dissipates via charge-recombination reactions in a non-assimilatory process.

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Such a hypothesis on the regulation of photodamage by the redox state of QA requires that a linear increase of the rate constant of photodamage (Fig. 3) must then underlie a linear increase in the fraction of reduced QA as a function of irradiance. Results in the literature (summarized in Fig. 5) show that such a relationship does exist. In experiments with a higher plant (barley) and a green alga (Chlorella vulgaris), the fraction of reduced QA increases linearly as a function of light intensity, especially in the low light intensity region where photosynthesis is far from being saturated. Interestingly, the linear relationship between the fraction of reduced QA and irradiance extends well beyond the light intensity at which photosynthesis saturates. For example, in C. vulgaris (Fig. 5), I₅₀ is reached at ~180 µmol photons m⁻² s⁻¹, however, the linearity in the 'fraction of reduced QA versus irradiance' is maintained for light intensities greater than 600 µmol photons m⁻² s⁻¹. A similar observation was made in experiments with Hordeum vulgare (Fig. 5). This quantitative discrepancy in the light-saturation curve of photosynthesis and the fraction of reduced QA is not understood.
The steady-state oxidation-reduction level of QA on the acceptor side of PSII might be the common denominator to many physiological and environmental conditions that modulate the rate of PSII photodamage in chloroplasts. In general, according to this hypothesis, conditions that limit the rate of photosynthesis, or enhance the rate of light absorption relative to electron transport, would cause an over-reduction of the plastocyanine pool\(^4\). This condition would shift the redox state of QA from oxidized to reduced, thereby increasing the probability of photodamage. Higher light intensities, a larger chlorophyll antenna size, inorganic carbon limitation or suboptimal temperature will tend to shift the redox state of QA from oxidized to reduced in the course of steady-state photosynthesis, excitation energy is utilized in electron transport. When QA is reduced in the course of steady-state photosynthesis, excitation energy is dissipated by non-assimilatory ‘charge recombination’ processes. The latter might lead to a generation of long-lived excited states of chlorophyll which, in the presence of oxygen, can cause irreversible photodamage to D1. The picture emerging, therefore, is that physiological and environmental parameters modulate the redox state of QA, which in turn defines the photochemical or non-assimilatory dissipation of excitation energy and, thus, the low or high probability of photodamage in the PSII reaction center complex.

Conclusions

Results in the literature support the theory that the probability for PSII photodamage depends on the redox state of QA. Photodamage will occur with a low probability when QA is oxidized and excitation energy is utilized in electron transport. When QA is reduced in the course of steady-state photosynthesis, excitation energy is dissipated by non-assimilatory ‘charge recombination’ processes. The latter might lead to a generation of long-lived excited states of chlorophyll which, in the presence of oxygen, can cause irreversible photodamage to D1. The picture emerging, therefore, is that physiological and environmental parameters modulate the redox state of QA, which in turn defines the photochemical or non-assimilatory dissipation of excitation energy and, thus, the low or high probability of photodamage in the PSII reaction center complex.

References


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**Fig. 5.** The fraction of reduced primary quinone acceptor (QA) as a function of light intensity in Chlorella vulgaris (filled circles), reproduced, with permission, from Ref. 41, and barley (Hordeum vulgare, unfilled circles), reproduced, with permission, from Ref. 40. The fraction of reduced QA was measured as (1–q\(P\)) where q\(P\) is the coefficient of photochemical fluorescence quenching\(^3\). The light intensity for the saturation of photosynthesis (I\(s\)) in these samples was ~180 and ~200 \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\) for Chlorella and barley, respectively\(^4\).

**Fig. 6.** Model depicting the relationship between the probability for photosystem-II (PSII) photodamage, the redox state of the primary quinone acceptor (QA) and light intensity, carbon availability, PSII chlorophyll (Chl) antenna size and temperature. D1 is the reaction center protein.


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