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 reduced, 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[1]. 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[2,3]. 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[4–6]. 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 the PSII repair process.
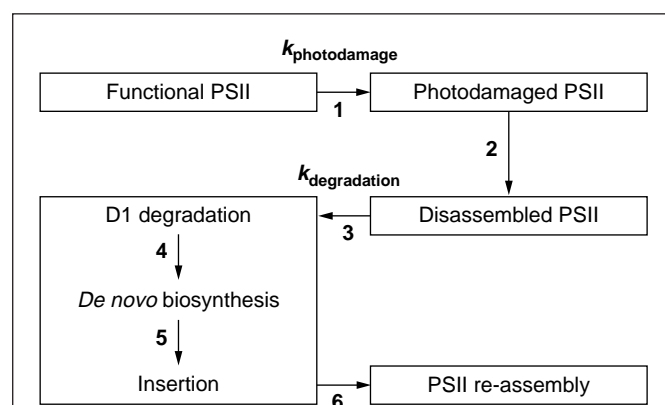

Fig. 1. Temporal sequence of events in photosystem-II (PSII) damage, holocomplex disassembly, and degradation and replacement of the D1/32 kDa reaction center protein. The rate constant of photodamage ($k_{photodamage}$) depends on the incident light intensity[19–21]. The rate of PSII disassembly (step 2) is not limiting under a broad range of incident intensities. Direct D1 degradation ($k_{degradation}$) and *de novo* D1 biosynthesis (step 4) become rate limiting under high light intensities. The rate of D1 degradation is the overall rate-limiting step of the PSII 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[5,7]. This condition is known as photoinhibition of photosynthesis. It causes significant losses in plant growth and productivity.

Photosystem-II damage and repair cycle

In broad terms, photodamage to D1 is followed by:

- Prompt, partial disassembly of the PSII holocomplex.
- Exposure of the photodamaged 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[4].

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 photodamage exceeds the capacity for repair.

Photodamage to PSII occurs in the light with a half time ranging from 8 h to 30 min (Ref. 7; Fig. 1), depending on light intensity and the functional status of the photosynthetic apparatus. In the cascade of reactions that constitute the repair process, the rate-limiting step is the degradation or replacement of photodamaged D1 (Fig. 1), which reportedly occurs with a half time of 60 ± 15 min (Refs 8,9).
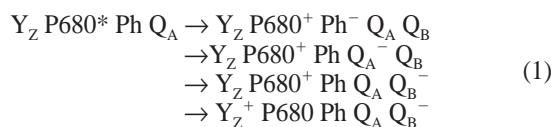
The temporal sequence of events leading to the recovery of the photosynthetic apparatus from photodamage is consistent with the known properties of frequent D1 turnover in chloroplasts[10] and also consistent with the heterogeneity observed in the configuration and function of PSII (Ref. 11). On the one hand, our limited knowledge does not include information about the identity and regulation of enzymes that perform the PSII repair or information about the genes that code for the repair proteins. On the other hand, recent work has provided information on the modulation of PSII photodamage by physiological and environmental conditions, once again raising the question about the mechanism and the regulation of photodamage under *in vivo* conditions.

Mechanism of D1 photodamage

An explanation of the adverse photodamage event at PSII can be provided upon consideration of the oxidation–reduction reactions that take place in the D1/D2 heterodimer. For the purposes of this review, these can be divided into steady-state electron-transfer reactions – when the primary quinone acceptor Q_A is oxidized or when Q_A is reduced.

Electron-transfer reactions when the primary quinone acceptor is oxidized

When the plastoquinone pool and Q_A are oxidized, the light absorption and the ensuing photochemical charge separation in the reaction-center proteins leads to forward electron transport from water to plastoquinone (Eqn 1).
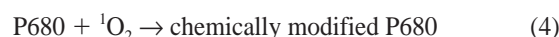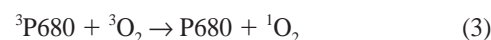
$$\begin{aligned} Y_Z\ P680^*\ Ph\ Q_A &\rightarrow Y_Z\ P680^+\ Ph^-\ Q_A\ Q_B \\ &\rightarrow Y_Z\ P680^+\ Ph\ Q_A^-\ Q_B \\ &\rightarrow Y_Z\ P680^+\ Ph\ Q_A\ Q_B^- \\ &\rightarrow Y_Z^+\ P680\ Ph\ Q_A\ Q_B^- \end{aligned} \quad (1)$$

where P680 is the photochemical reaction center chlorophyll molecule in D1, Ph is pheophytin, Y_Z is tyrosine, Q_A is the stably bound primary quinone and Q_B the reversibly bound secondary quinone electron-acceptor molecule. Q_B can be any plastoquinone molecule temporarily bound in the herbicide or Q_B binding site of D1.

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 400–600 µs). On the donor side of PSII, the positive charge on $P680^+$ is neutralized by electron-transfer from tyrosine Y_Z (reaction half time of 20–200 ns)[12]. 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_2O molecules, the release of four electrons, four protons and of molecular O_2. This set of reactions constitutes a successful conversion of excitation energy into chemical potential.

Electron-transfer reactions when Q_A is reduced

When Q_A is reduced at the time 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[13]. The electron transfer and excitation dissipation events that occur in the PSII reaction center under these conditions are summarized in Eqns 2–4.

$$P680^*\ Ph\ Q_A^- \rightarrow P680^+\ Ph^-\ Q_A^- \rightarrow {}^3P680\ Ph\ Q_A^- \quad (2)$$

$$^3P680 + {}^3O_2 \rightarrow P680 + {}^1O_2 \quad (3)$$

$$P680 + {}^1O_2 \rightarrow \text{chemically modified P680} \quad (4)$$

$$P680^+ + {}^1O_2 \rightarrow \text{chemically modified } P680^+ \quad (5)$$

Excitation energy in P680 generates a photochemical charge separation (step 1 in Eqn 2). In the absence of electron transfer from Ph^- to Q_A (Q_A is already reduced), the $P680^+\ Ph^-$ configuration is arrested for relatively long periods of time (tens of ns)[14], eventually promoting a recombination of the positive and negative charges in the $P680^+\ Ph^-$ pair. This charge recombination reaction has a high probability of generating P680 triplet[15] (step 2 in Eqn 2). The P680 triplet is quenched efficiently by $^3O_2$ (Ref. 16), thereby generating the highly reactive singlet oxygen ($^1O_2$; Ref. 17; Eqn 3). The lifetime of $^1O_2$ in hydrophobic environments, such as the microenvironment in the vicinity of P680, is estimated to be in the 10–30 µs range[18], that is, sufficiently long to permit an adverse reaction between $^1O_2$ and either P680 or $P680^+$ (Eqns 4 and 5, respectively).

Light absorption and utilization by the photosynthetic apparatus

The light-saturation curve of photosynthesis defines the characteristics, or 'vital signs', of a plant tissue. In this important measurement, the rate of O_2 evolution, or CO_2 assimilation, is plotted as a function of the probing actinic light intensity. In such a photosynthesis versus light intensity curve (Fig. 2), the rate of photosynthesis 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 $>I_S$. This light-saturated rate (P_{max}) provides a measure of the capacity of photosynthesis for the leaf or algal sample.

It is evident that light absorption by the photosynthetic apparatus will increase linearly with light intensity whereas the rate of photosynthesis saturates at I_S. Thus, at light intensities greater than those required for the saturation of photosynthesis, plants,

algae and cyanobacteria will encounter an imbalance between the processes of light absorption and utilization, the magnitude of which will depend on the incident light intensity and on the photosynthesis saturation intensity I_S. For example (Fig. 2), at light intensities $>I_S$ (~250 µmol photons m^{-2} s^{-1}) the photosynthetic apparatus will absorb photons that cannot be efficiently utilized in the process of oxygen production or CO_2 fixation. The excess photons 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 $<I_S$ and become significant at light intensities $>I_S$. 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)[19] and in a green alga (*Dunaliella salina*)[20]. 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 illumination for PSII inactivation, indicating that PSII photodamage depends on the total number of photons absorbed and not on the rate of photon absorption *per se*[21]. 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 indicates that the rate of photodamage is dependent on the ability of PSII to intercept electromagnetic radiation[22,23]. The chlorina F2 mutant has a PSII antenna size of only 50 Chl *a* molecules, compared with the 250 Chl (*a* and *b*) found in the wild type[24]. Under identical incident light intensities, the Chl *b*-less chlorina F2 mutant sustained slower rates of photodamage than the corresponding wild type[22]. It has also been reported that photoinhibition is totally independent of the PSII light-harvesting Chl antenna size[25,26]. Re-examination of this question[27,28] 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 PSII 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 accentuate photoinhibition in cyanobacteria[29] and higher plants[30,31]. It has also been reported that electron transport to oxygen via the photorespiratory oxidase[32] or the Mehler reaction[33] can protect against photoinhibition in pea leaves (but see Ref. 34). However, antisense transgenic plants with a substantially lower cytochrome b_6–f complex content, in which illumination produced slow rates of linear electron transport and in which Q_A accumulated in the reduced state, did not show the expected increase in their susceptibility to photoinhibition[35,36]. 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_{max} of ~100 pmol O$_2$ (10^6 cells)$^{-1}$ s^{-1}], or with 3% CO$_2$ in air, bubbled into the culture [P_{max} of ~250 pmol O$_2$ (10^6 cells)$^{-1}$ s^{-1}]. These conditions differed by a factor of ~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'.

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 ~250 µmol photons m^{-2} s^{-1}. The light-saturated rate (P_{max}) of this sample was ~160 mmol O$_2$ (mol Chl)$^{-1}$ s^{-1}.

Fig. 3. Irradiance dependence of the rate constant for photosystem-II (PSII) photodamage. The first-order rate constants $k_{photodamage}$ were measured in the presence of lincomycin from the half-time of D1 (the reaction center protein) decay traces in *Dunaliella salina* (filled circles) or fluorescence ($F_\mathrm{v}/F_\mathrm{max}$) decay traces in *Cucurbita pepo* (pumpkin, unfilled circles) according to the equation $k_{photodamage} = \ln 2/\tau$, where τ is the half-time of the decay kinetics in question. (Reproduced, with permission, from Ref. 20 and Ref. 19 for *D. salina* and pumpkin, respectively.) Note the difference, by a factor of ~2, in the slope of the two $k_{photodamage}$ versus I lines. This difference is attributed in part to the larger PSII chlorophyll antenna size of *D. salina* than pumpkin, and in part to the gradients of light intensity forming within higher plant leaves[44]. The latter attenuates the average light intensity to which the leaf is exposed.

Fig. 4. Effect of photosystem-II (PSII) light-harvesting chlorophyll (Chl) antenna size, carbon source and rate of photosynthesis on the rate constant of photodamage ($k = \ln 2/\tau$, where τ is the half-time of photodamage) in *Dunaliella salina*. The rate of photodamage was measured at an incident intensity of 2000 mmol photons m^{-2} s^{-1} in low-density cultures. Cells with 'Large' Chl antenna size had, on the average, an ~3.5-fold greater PSII Chl antenna size and ~1.5-fold greater PSI Chl antenna size than cells with 'Small' Chl antenna size. (a) Cells were grown in the presence of an initial 25 mM $NaHCO_3$ as the sole carbon source in the medium (black bars). (b) Cells were supplemented with 3% CO_2 in air, bubbled in the culture, as the main inorganic carbon source (gray bars). In light-shift experiments ($NaHCO_3$ grown cells to CO_2-bubbling conditions), the rate of photosynthesis was enhanced by up to 60% of that measured in the presence of $NaHCO_3$.
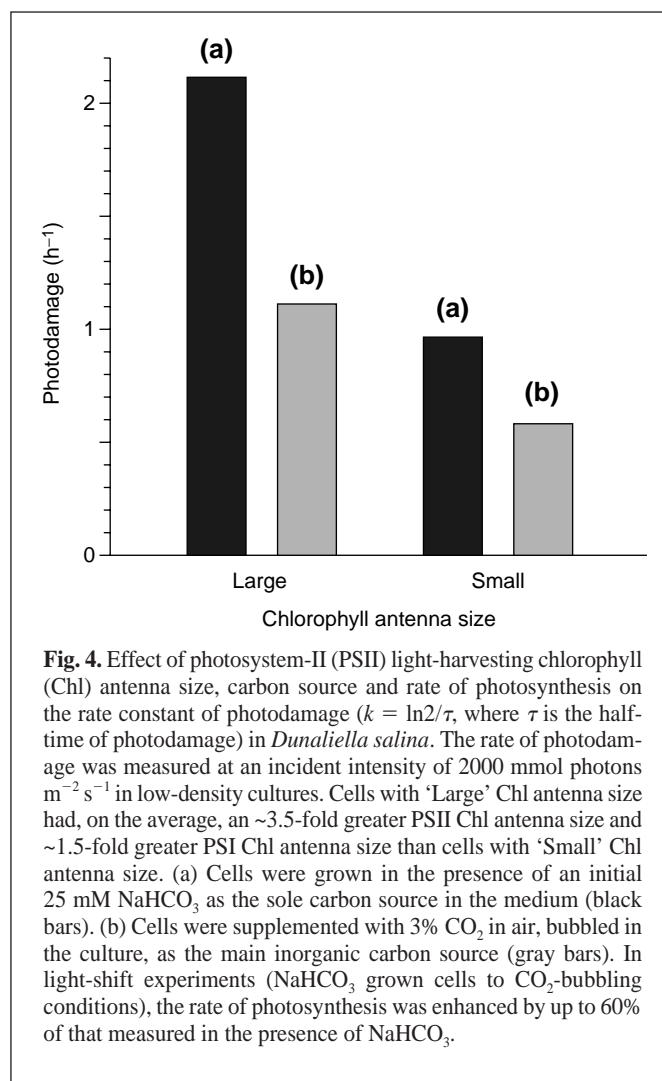
Suboptimal temperature accentuates photodamage and photoinhibition

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[37]. Suboptimal temperatures also enhance the rate of photodamage as they slow down electron transport and shift the steady-state redox level of Q_A in chloroplasts. A single, curvilinear relationship exists between the steady-state redox level of Q_A and the susceptibility of photosynthesis to photoinhibition, a phenomenon that is independent of the wheat or rye cultivars examined[38]. This suggests that low temperature imposes constraints on the biochemical steps of photosynthesis, resulting in an increased proportion of closed PSII reaction centers (reduced Q_A) in thylakoids[38], a condition that leads to greater rates of photodamage. Thus, the rate of photodamage in terrestrial plants, green algae and cyanobacteria does not respond to changes in growth irradiance or growth temperature *per se*, but rather responds to changes in the steady-state redox level of Q_A in chloroplasts[39]. These results are also inconsistent with a PSII 'photon counter'.

Redox state of the PSII primary quinone acceptor Q_A defines the probability of D1 photodamage

Altogether, the results presented here are not consistent with the concept of a 'photon counter' in PSII. On the contrary, they suggest that the probability of photodamage might be totally different in the two redox states of PSII (the primary quinone acceptor Q_A being in the oxidized or reduced form during steady-state illumination). More specifically, they suggest that there is a low inherent probability for photodamage when Q_A is oxidized and excitation energy dissipates by useful photochemistry in the form of linear electron transport through PSII. Conversely, there is a significantly higher probability for photodamage when Q_A 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 Q_A requires that a linear increase of the rate constant of photodamage (Fig. 3) must then underline a linear increase in the fraction of reduced Q_A 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)[40] and a green alga (*Chlorella vulgaris*)[41,42], the fraction of reduced Q_A 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 Q_A and irradiance extends well beyond the light intensity at which photosynthesis saturates. For example, in *C. vulgaris* (Fig. 5), I_S is reached at ~180 μmol photons m^{-2} s^{-1}, however, the linearity in the 'fraction of reduced Q_A' 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 'fraction of reduced Q_A' is not understood.
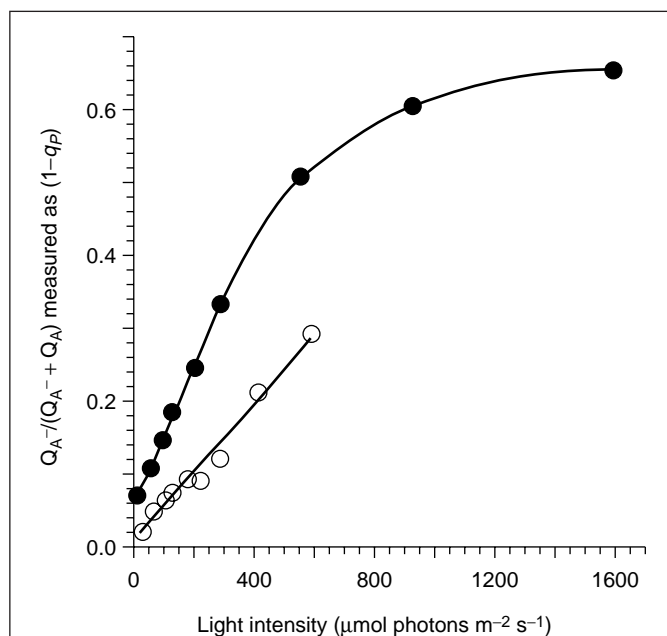
Fig. 5. The fraction of reduced primary quinone acceptor (Q_A) as a function of light intensity in *Chlorella vulgaris* (filled circles), reproduced, with permission, from Ref. 41, and barley (*Hordeum vulgare*; unfilled circles), reproduced, with permission, from Ref. 40. The fraction of reduced Q_A was measured as $(1-q_P)$ where q_P is the coefficient of photochemical fluorescence quenching[45]. The light intensity for the saturation of photosynthesis (I_S) in these samples was ~180 and ~200 μmol photons m^{-2} s^{-1} for *Chlorella* and barley, respectively[40,41].

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

The steady-state oxidation–reduction level of Q_A 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 plastoquinone pool[43]. This condition would shift the redox state of Q_A 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 Q_A from the oxidized to reduced. The converse is also true (Fig. 6). The probability of photodamage is estimated to be at least one order of magnitude greater when Q_A is in the reduced rather than the oxidized state (A. Melis, unpublished).

Conclusions

Results in the literature support the theory that the probability for PSII photodamage depends on the redox state of Q_A. Photodamage will occur with a low probability when Q_A is oxidized and excitation energy is utilized in electron transport. When Q_A 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 Q_A, 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.

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