

A photoprotective role for O₂ as an alternative electron sink in photosynthesis?

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Photoprotection of the photosynthetic apparatus has two essential elements: first, the thermal dissipation of excess excitation energy in the photosystem II antennae (i.e. non-photochemical quenching), and second, the ability of photosystem II to transfer electrons to acceptors within the chloroplast (i.e. photochemical quenching). Recent studies indicate that the proportion of absorbed photons that are thermally dissipated through the non-photochemical pathway often reaches a maximum well before saturating irradiances are reached. Hence, photochemical quenching is crucial for photoprotection at saturating light intensities. When plants are exposed to environmental stresses and the availability of CO₂ within the leaf is restricted, the reduction of oxygen by both the photorespiratory and the Mehler ascorbate peroxidase pathways appears to play a critical photoprotective role, substituting for CO₂ in sustaining electron flow. Induction of high activity of the Mehler ascorbate peroxidase pathway may be associated with acclimation to environmental stress.

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Abbreviations

MDA monodehydroascorbate

PSII photosystem II

rubisco ribulose-1,5-bisphosphatase carboxylase/oxygenase

Introduction

On sunny days, leaves at the top of canopies encounter light intensities that exceed their photosynthetic capacity. It appears that evolution has refined the photosynthetic apparatus with an emphasis on high efficiency in limiting light with regulatory features that help to ensure that high intensities can be endured without the accumulation of photodamage. Although this view is somewhat over simplified, it is true that when irradiances are high, other factors, such as the maintenance of leaf water status, often take physiological precedence over maximizing photosynthesis [1]. Thus, the regulation of leaf photosynthesis is usefully viewed as a dynamic balancing act in which photoprotection is reversibly traded for photosynthetic efficiency [2]. The ΔpH-dependent thermal dissipation of absorbed light is a centrally important element of photoprotection. Nevertheless, overall photoprotection is made up of a diverse set of processes that can include:

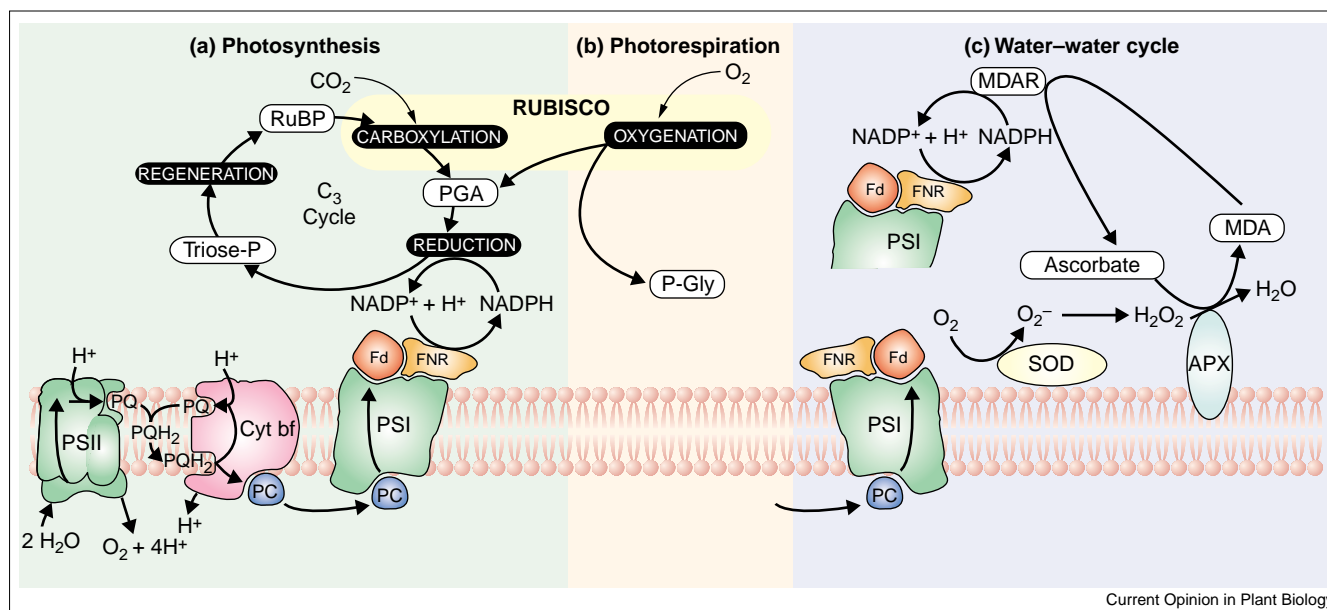
strategic leaf and chloroplast movements, detoxification of photosynthetically produced reactive molecules via intricate biochemical pathways, a variety of repair processes to prevent the accumulation of photodamage, and the utilization of excess absorbed light when CO₂ is limiting by an array of alternative electron acceptors [3]. Considerable attention has been focussed on the mechanisms involved in quenching excess excitation energy in pigment–protein antennae complexes that are associated with thylakoid photosynthetic apparatus [2,4,5]. Rather less consideration has been given to the role of potential alternative sinks for electrons, of which O₂ is the most prominent candidate. In this review, we examine the premise and evidence for the importance of O₂ as an alternative electron acceptor to CO₂ in protecting leaves from photodamage.

Regulation of electron flux

In considering the role of O₂ in protecting the photosynthetic apparatus by utilizing excitation energy that exceeds the capacity of CO₂ assimilation, it is useful to examine factors that regulate electron flux in leaves under physiological conditions. It is now common practice to monitor the relative rates of electron fluxes in leaves by non-invasive measurement of chlorophyll fluorescence. This method allows researchers to estimate the relative quantum efficiency of electron transport through photosystem II (PSII), commonly termed the PSII operating efficiency. Two factors determine the PSII operating efficiency. First, the efficiency with which excitation energy is transferred to photochemically active (i.e. open) PSII reaction centers, which is determined by the rate of thermal dissipation of excitation energy in the PSII antennae (i.e. non-photochemical quenching). Second, the ability of PSII to transfer electrons to acceptors (i.e. photochemical quenching), which is determined by the availability of CO₂ or other suitable electron sinks within the chloroplast [6]. The relative importance of these two quenching components in regulating the operating efficiency of PSII in leaves is not fixed; it can be influenced directly and indirectly by conditions both within and outside of the leaf. The amount of light incident on a leaf can be one of the important factors that influences the balance between photochemical and non-photochemical quenching processes.

Many published studies have provided evidence that changes in both non-photochemical and photochemical quenching can contribute to the reductions in the PSII operating efficiency that occur as steady-state photosynthesis is attained. On closer examination, the data frequently show that increases in non-photochemical quenching primarily occur at light intensities well below those at which photosynthesis is saturated. Furthermore,

Figure 1



This schematic drawing of a chloroplast depicts alternative electron acceptor pathways that may play an important role in plant photoprotection. **(a)** Photosynthetic carbon reduction is shown as a three-stage cycle. Carboxylation: rubisco catalyzes the covalent linkage of a molecule of CO_2 to phosphoglyceric acid (PGA). Reduction: energy in the form of ATP and NADPH is used to form the simple carbohydrate Triose-P. Regeneration: energy in the form of ATP is used to regenerate ribulose 1,5-bisphosphate (RuBP) for carboxylation. **(b)** The full photorespiratory carbon oxidation cycle of C_3 plants involves three separate plant cell organelles. The initial production of phosphoglycolate (P-Gly) by the rubisco-catalyzed oxygenation of RuBP is shown here. A phosphatase produces glycolate, which leaves the chloroplast via a specific envelope transporter *en route* to the

peroxisome (not shown). **(c)** The water–water cycle. A superoxide radical anion (O_2^-) is produced when molecular oxygen (O_2) is reduced by ferredoxin (Fd). The superoxide radicle is disproportionated by thylakoid-membrane-attached superoxide dismutase (SOD) to produce hydrogen peroxide (H_2O_2). This H_2O_2 is reduced by ascorbate to form water in a reaction catalyzed by ascorbate peroxidase (APX). Monodehydroascorbate (MDA) is produced in this reaction, which provides yet another sink for electrons as ascorbate is regenerated with electrons from ferredoxin in a reaction mediated by monodehydroascorbate reductase (MDAR). Cyt bf, cytochrome bf complex; FNR, ferredoxin-NADP reductase; PC, plastocyanin; PQ and PQH_2 , plastoquinone and reduced plastoquinone; PSII and PSI, photosystem II and I.

reductions in photochemical quenching primarily determine the decreased quantum efficiency of electron transport at higher light levels. A striking example was provided by a recent study of the photosynthetic efficiencies of stomatal guard cells and mesophyll cells in *Tradescantia* leaves [7•]. In these cells, significant increases in non-photochemical quenching were observed only up to a photosynthetic photon flux density (PPFD) of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas large decreases in photochemical quenching determined the photosynthetic efficiency at higher light levels [7•]. Similarly, the large damping oscillations that are observed in the PSII operating efficiency of mature maize leaves during the induction of CO_2 assimilation following a dark-to-light transition are almost entirely attributable to fluctuations in the ability of PSII to transfer electrons to available acceptors [8]. Only very small changes in non-photochemical quenching occur [8].

Thus, it appears that the proportion of absorbed photons that are thermally dissipated through the non-photochemical pathway can reach a maximum at moderate light levels. Although the total flux of energy through the non-photochemical pathway continues to increase in proportion to

light intensity, the ability of the non-photochemical pathway to limit the reduction of the primary quinone acceptor of PSII decreases as light intensities reach levels at which photosynthesis is saturated and above. Even so, we know that in a normal healthy leaf in which CO_2 is adequately available, the quinone acceptors of PSII remain partially oxidized in full sunlight (i.e. photochemical quenching does not fall to zero) because of the active transfer of electrons to available electron sinks. However, when CO_2 availability is restricted, as it is daily expected to be for fully sun-exposed leaves, the contribution of electron acceptors other than CO_2 , especially of O_2 , must increase to maintain the partial oxidation of the PSII acceptors and thereby counter the photoinactivation of PSII.

Photorespiration

In C_3 leaves, the photorespiratory oxygenation of ribulose-1,5-bisphosphate by rubisco (ribulose-1,5-bisphosphatase carboxylase/oxygenase) (Figure 1) constitutes a major alternative sink for electrons [9,10•]. Rubisco catalyses a competitive reaction in which oxygen is favored over CO_2 as a substrate as temperature increases or as the intercellular CO_2 concentration declines when stomatal conductance is

low. Under ambient atmospheric conditions at 25°C, about 20% of the total electron flux through rubisco is diverted to oxygen. However, a reduction of ambient intercellular CO₂ concentration by about 80 μmol mol⁻¹ would change the carboxylation to oxygenation stoichiometry from 4:1 to about 3:1. Even though photorespiration dissipates no more energy than photosynthesis on a molar basis [11], it seems indisputable that the substitution of O₂ for CO₂ contributes meaningfully to maintaining the partial oxidation of the PSII acceptors when CO₂ availability is restricted because of partial stomatal closure. Perhaps this role for oxygenation has been a contributing factor in constraining the evolution of higher specificity factors for rubisco.

Oxygen and monodehydroascorbate reduction (the water–water cycle)

In principle, oxygen reduction could sustain significant levels of photosynthetic electron flux not only through its role in photorespiration but also by its direct reduction by PSI [12]. Although the direct reduction of oxygen would be an effective strategy to increase the ratio of ATP/NADPH to match the requirements of carbon reduction, as well as to sustain photoprotective electron flux when CO₂ is scarce, it nevertheless carries hazards inherent in the formation of reactive oxygen species. Superoxide radicals generated by the one-electron reduction of molecular oxygen by photosystem I are rapidly converted within the chloroplast to hydrogen peroxide by CuZn-superoxide dismutase [13]. Whereas hydrogen peroxide associated with the photorespiratory pathway is generated and detoxified by catalase in peroxisomes, the hydrogen peroxide produced in the chloroplast is detoxified almost exclusively by ascorbate peroxidase. The reduction of oxygen by PSI is accompanied by the production of monodehydroascorbate (MDA). The subsequent reduction of MDA via ferredoxin doubles the electron flux associated with this oxygen reduction pathway (Figure 1). In fact, because MDA is also produced in the chloroplast by several other photoreactions (e.g. the reduction of tocopherol), the electron flux supporting the ferredoxin-dependent reduction of MDA would be expected to be greater than the electron flux for the photoreduction of oxygen itself.

It has been suggested that the photoreduction of O₂ to water by the Mehler ascorbate peroxidase pathway (Figure 1) in intense light can involve 30% of the total electron flux [14]. The extremely high concentrations of ascorbate (i.e. 20–300 mM) [15•] and of glutathione (i.e. 25 mM), a key metabolite for the regeneration of ascorbate from MDA [14], found in chloroplasts appear to be sufficient to sustain the rapid turnover of the water–water cycle [14,16•]. The size of the ascorbate pool in leaves is also controlled by light intensity. The conversion of *L*-galactono-1,4-lactone to ascorbate, the last step in ascorbate biosynthesis, is enhanced by high light and occurs faster in leaves that are acclimated to high light [17]. Mutants that are deficient in ascorbate biosynthesis

function normally until exposed to high light, suggesting that high levels of ascorbate are required to deal with photooxidative stress [17]. If the regeneration of MDA does not keep pace with the rate of oxygen reduction, as appears to be the case for chilling-sensitive plants that are illuminated at cool temperatures, the overall redox status of the chloroplast declines and inhibits photosynthesis [18,19].

Unfortunately, it is difficult to measure directly the rate of electron transport to O₂. In fact, less than 10% of the more than 100 recent papers discussing this reaction actually report measurements of the flux. To date, there is no direct proof that a water–water cycle operates at high rates when CO₂ assimilation is restricted. However, indirect estimates of water–water cycle activity that have been obtained from gas exchange and fluorescence measurements on leaves indicate significant water–water cycle activity [20]. This activity increases in leaves experiencing both high light intensities and low internal leaf CO₂ concentrations, a condition that suppresses rubisco activity [20]. On the other hand, direct mass spectrometric measurements of ¹⁶O₂ and ¹⁸O₂ exchange in tobacco with transgenetically reduced rubisco levels did not show the expected increase in the activity of the water–water cycle [21,22••]. These elegant experiments are designed to test the capacity of the water–water cycle to replace the rubisco-dependent reduction of CO₂ and O₂. Nevertheless, their interpretation should be tempered by our overall lack of knowledge about conditions that may be necessary to induce or activate the water–water cycle, conditions that may not be satisfied by the transgenic restriction of electron flux to CO₂.

The responses of leaves to certain environmental stress conditions seem to provide persuasive evidence that the water–water cycle is important in protecting the chloroplasts from photooxidative damage. Dehydration induces an increase in O₂ uptake into wheat leaves, which has been attributed to increased activity of the water–water cycle activity [23]. When maize leaves are exposed to chilling temperatures in the field, a large increase in the ratio of PSII electron transport to CO₂ assimilation has been observed [24]. Measurements of electron flux through PSII by chlorophyll fluorescence procedures and CO₂ reduction by infrared gas analysis provided evidence that as many as 21 electrons were transported through PSII for each CO₂ molecule assimilated in these chilled maize leaves, compared with six electrons in unstressed leaves [24]. This surfeit of electron flux relative to CO₂ reduction occurs simultaneously with an increase in the activities of essentially all of the enzymes that are involved in scavenging for reactive oxygen species and in ascorbate regeneration, as well as with an increase in the level of ascorbate itself [24]. Similar measurements on mangrove leaves under field conditions in tropical Australia showed that electron fluxes through PSII were more than three times greater than could be accounted for by carbon assimilation [25]. A new approach, coupling highly sensitive measurements of differential oxygen [26] with estimates of PSII flux

obtained by measuring chlorophyll fluorescence was used recently to monitor changes in O_2 consumption by the Mehler ascorbate peroxidase pathway. Preliminary measurements on the leaves of mango trees showed that exposure to chilling temperatures induced a light-dependent pathway for O_2 consumption that consumed 50% of the total electron flux (DJ Allen, DR Ort, abstract 640, Plant Biology 2001, Providence, RI, July 2001. <http://abstracts.aspb.org/aspp2001/public/P43/0570.html>).

The high activity of the water–water cycle when CO_2 assimilation is restricted implies either that sinks for the ATP produced by proton-coupled electron flux from water to O_2 must exist or that there is a mechanism for uncoupling the electron flux from ATP synthesis. Otherwise, the elevated trans-thylakoid proton electrochemical differences would restrict electron flux and would be expected to lead to photoinactivation and damage to the PSII reaction centers. Although the uncoupling of electron flux from ATP synthesis has not been researched directly in this context, the ‘slippage’ of the chloroplast ATP synthase at high ΔpH values has been demonstrated several times; it allows a sizeable leakage of protons through the ATP synthase without the formation of ATP [27–30].

Chlororespiration

There is now strong evidence that a chlororespiratory pathway operates in the chloroplasts of higher plants [31]. The evidence suggests that this pathway involves the dark reduction of plastoquinone in the thylakoid membrane by NADPH or NADH. The subsequent oxidation of plastoquinol ultimately terminates with the reduction of molecular O_2 . A considerable amount of current research is investigating the identities of the chlororespiratory plastoquinone reductase [32,33] and oxidase [34–36], and is seeking to determine whether the chlororespiratory oxidase is localized to the chloroplast [37]. The existence of a chlororespiratory plastoquinol oxidase suggests the existence of yet another pathway through which electrons from PSII might be diverted to O_2 . However, the overall electron-flux capacity of this pathway is very low (approximately 0.3% of light-saturated photosynthetic electron flux [38]), and so it would not contribute appreciably to removing electrons from PSII or in providing photoprotection. In addition, the electron flux into plastoquinone from the chlororespiratory reductase would be insufficient to compete with photochemical oxidation by photosystem I, and therefore would not be expected to cause any decrease in photochemical quenching (because of the reduction of the PSII quinone acceptor via redox equilibration with the plastoquinone pool) [39].

Conclusions

The thermal dissipation of a large proportion of excess excitation energy in the PSII antennae (i.e. non-photochemical quenching) and the ability of PSII to transfer electrons to acceptors (i.e. photochemical quenching) are two fundamentally important elements of photoprotection

in chloroplasts. Close examination of a range of published data indicates that the proportion of absorbed photons that are thermally dissipated through the non-photochemical pathway often reaches a maximum well before saturating irradiances are reached. Furthermore, at saturating light intensities, the photochemical pathway plays a critical role in maintaining the partial oxidation of the PSII acceptors that is required for photoprotection.

A linear relationship between electron flux through PSII and rate of CO_2 assimilation has been widely confirmed in healthy unstressed leaves of numerous plant species in which photorespiration is absent (in the case of C_4 plants) or suppressed (under low O_2 concentrations) [40]. When photorespiration does occur, O_2 sustains a substantial electron flux from PSII that becomes larger as CO_2 is drawn down within the leaf and as leaf temperature increases due to lowered stomatal conductance. The direct reduction of O_2 by photosystem I and the regeneration of ascorbate, which is required for sustained rapid turnover of the water–water cycle, are other potentially large alternative electron sinks that contribute to photoprotection. These alternative electron sinks are particularly important in plants growing for extended periods under a variety of stress conditions that restrict the availability of CO_2 within the leaf because of reduced stomatal conductance. In such situations, PSII electron fluxes can be substantially in excess of the requirements for carbon assimilation and photorespiration. Such imbalances are often coupled to increases in the activities of enzymes that are involved in scavenging for reactive oxygen species and in ascorbate regeneration, implying that O_2 is substituting (via the Mehler ascorbate peroxidase pathway) for CO_2 and photorespiration in sustaining electron flow.

Substantial evidence is mounting to show that O_2 plays a substantive role as an alternative electron acceptor in photoprotection, particularly in plants that have acclimated to long-term environmental stresses under high light intensities. However, the initial interpretations of well-designed and well-executed studies, such as the transgenic depletion of rubisco, are not easily reconciled with such a prominent role for O_2 . Nevertheless, it is quite possible that without acclimation to environmental stresses, the rapid turnover of the water–water cycle is not possible in the transgenic plants in these studies. There has been considerable recent progress in understanding mechanisms for the detection of stresses, including excess light, and the signal transduction pathways involved in engaging acclimatory responses [41,42]. More direct measurements of actual oxygen fluxes are needed to gain better insights into conditions that may regulate the induction or activity of the water–water cycle. In addition, the roles that might be played by other alternative acceptors such as nitrate, sulfate, the light-stimulated synthesis of fatty acids [43] or oxaloacetate reduction [44] must be investigated further before an overall conceptual view of alternative-acceptor-dependent photoprotection will emerge.

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