When There Is Too Much Light

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A great deal of importance has happened in research investigating photosynthetic response to environmental stress in the 25 years since the last anniversary issue of Plant Physiology. However, from my perspective, the importance of one set of discoveries stands out from the others for its far reaching influence on how we think about the photosynthetic response to a wide range on environmentally imposed limitations. As little as 15 years ago it was generally held that the success of plants in their environment was dictated by strategies that maximized the rate of photosynthesis. Further, maximum photosynthetic capacity was thought to be largely a static characteristic of individual leaves that was established during development. This view has now given way to the recognition that the regulation of photosynthesis in response to the environment is highly dynamic and dominated by a photoprotective process, the nonphotosynthetic thermal dissipation of absorbed light (4, 10, 14), which was entirely unknown at the time of Plant Physiology's 50th Anniversary. This brief overview describes what is currently understood about this centrally important photoprotective process and highlights areas of current inquiry that may presage a detailed mechanistic understanding in the near future.

MOST PLANTS ENCOUNTER EXCESS LIGHT CONDITIONS ON A DAILY BASIS

Most days plants encounter light intensities that exceed their photosynthetic capacity. Exactly what constitutes excess light for a leaf depends on its instantaneous environmental conditions and can vary over an exceedingly wide range of irradiance levels. For example, irrigated field-grown sunflower is typical of C3 crop plants, exhibiting maximum photosynthetic capacity during mid-morning with photosynthesis declining throughout the afternoon as stomatal conductance declines in response to declining leaf water potentials (21). Thus even under conditions which may not generally be considered stressful, stomatal conductance can substantially restrict CO_2 entry into leaves, rendering even moderate irradiances in the top of a crop canopy in excess of photosynthetic capacity.

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A DYNAMIC PROCESS ENABLING LEAVES TO REGULATE THERMAL DISSIPATION OF EXCESS ABSORBED LIGHT IS AT THE CENTER OF PLANT PHOTOPROTECTION

When environmental conditions prevent the maintenance of a high capacity for photosynthetic and photorespiratory carbon metabolism to utilize absorbed light, the likelihood for the photosynthetic generation of biologically damaging molecules including reduced and excited species of oxygen, peroxides, radicals, and triplet state excited pigments increases dramatically (1). Although some plants can reduce the amount of incident light that is absorbed through strategic leaf and chloroplast movements, rapid reduction in light absorption appears to play only a minor role in the challenge of coping with excess light.

The development of the techniques and biophysical interpretation of pulse modulated fluorescence in the mid-1980s by Bradbury and Baker (2) bolstered by important additions and refinements by many others (e.g. 7, 8, 20) provided the basis for a new understanding about the dynamic trade-off between photosynthetic efficiency and photoprotection (Fig. 1). A wide range of studies on many different species revealed that frequently over one-half of the light absorbed by photosystem II (PSII) chlorophylls in healthy, fully functional leaves can be redirected by a process that operates within the antenna ensemble of PSII, which harmlessly discharges excess photon flux energy as heat (3, 4, 10, 14). This thermal dissipation process is measured and often called nonphotochemical quenching, referring to the fact that the thermal dissipation of chlorophyll excited states competes with fluorescence emission as well as with photochemistry (i.e. photosynthesis).

ΔpH AND THE INTERCONVERSION OF XANTHOPHYLLS PLAY A CRITICAL ROLE IN REGULATING THERMAL ENERGY DISSIPATION IN PSII

Following the initial observations of Krause and Behrend (11) there is now a great deal of compelling evidence that excess light conditions are sensed or signaled by a large ΔpH (i.e. low-lumen pH), which forms when ATP utilization is restricted by CO_2 availability or by stress-induced dysfunction in the enzymology of carbon reduction (4, 10, 14). It is not

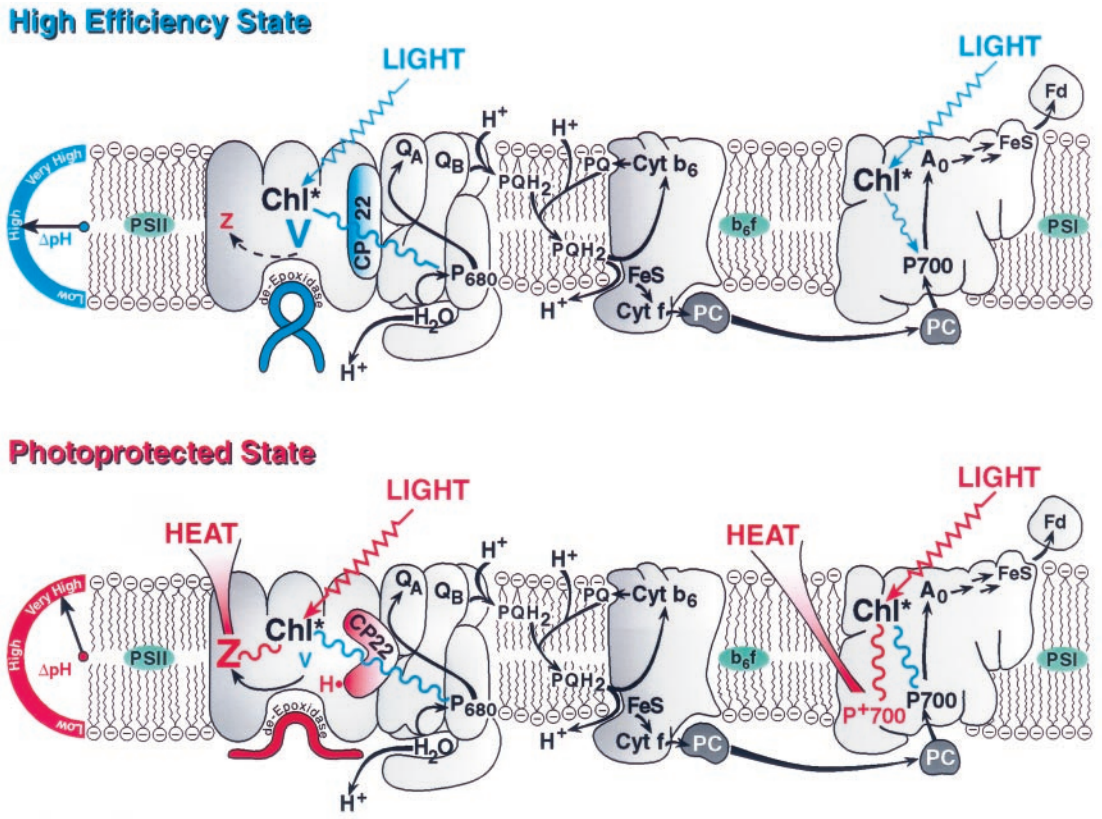

Figure 1. Model depicting the conversion of the thylakoid membrane at excess light from the high efficiency state (top) to the photoprotected state (bottom). The excess light condition is sensed by a very large ΔpH that initiates the non-photosynthetic thermal dissipation of absorbed light as described in the text. The major elements involved in the conversion between the high efficiency and photoprotected states are highlighted by the transition from blue to red. PSII, Photosystem II complex; PSI, photosystem I complex; b_6f, cytochrome b_6f complex; P_{680}, reaction center chlorophyll of PSII; Q_A and Q_B, quinone acceptors of PSII; PQ and PQH_2, plastoquinone and reduced plastoquinone; Cyt, cytochrome; FeS, Rieske iron sulfur protein; PC, plastocyanin; P_{700} and P_{700}^+, reduced and oxidized forms of the reaction center chlorophyll of PSI; A_o, primary acceptor of PSI; FeS, bound iron sulfur acceptors of PSI; Fd, soluble ferredoxin; Chl*, excited chlorophyll molecule; Z, zeaxanthin; V, violaxanthin; CP22, minor PSII pigment protein (also called PsbS) required for regulated thermal energy dissipation and believed to instigate protonation-dependent reorganization in LHCII.

always recognized, even by everyone working in this area of research, that ΔpH formation is exceedingly non-linear with light intensity (19). A ΔpH sufficient to drive net ATP synthesis (approximately 2.5 units) and thus photosynthetic CO_2 reduction is formed at 0.1% of full sunlight (15) and increases only on the order of 25% when the irradiance level is increased 1,000-fold. Thus only when the lumen pH is driven to very low values does photoprotective thermal energy dissipation within PSII become engaged.

Building on the ground breaking work of Yamamoto and colleagues (22), and Demmig-Adams, Björkman, and their coworkers (5), there is now a large body of experimental data supporting the notion that the low lumen pH activates violaxanthin de-epoxidase (4), which in turn converts violaxanthin, a xanthophyll pigment bound to the PSII light harvesting complex (LHCII), to zeaxanthin (and antheraxanthin). Thus, as depicted in Figure 1, zeaxanthin accumulates at the expense of violaxanthin under excess light initiating thermal energy dissipation. Well-characterized mutants of Arabidopsis lacking functional violaxanthin de-epoxidase are unable to engage photoprotective energy dissipation in PSII, pointing to an obligate role for zeaxanthin in this process in higher plants (14).

A second critical role of low lumen pH is the instigation of protonation-induced conformational change in one or more of the so-called minor LHC proteins of PSII. Although indirect evidence for several potential candidate LHCs has been reported, a recent breakthrough was made by Niyogi and colleagues showing that a deletion mutation in the gene encoding the minor PSII LHC PsbS (also called CP22) prevents thermal energy dissipation in PSII (12). Moreover, the mutation in PsbS also prevents an accompanying ΔpH- and zeaxanthin-dependent light scattering change that is thought to reflect a protonation-induced protein conformational change within PSII. The fact that this mutation in PsbS does not interfere with efficient light harvesting, water oxidation, or xanthophyll cycling supports a dedi-

cated role of this chlorophyll- and xanthophyll-binding protein in photoprotective energy dissipation rather than photosynthetic light harvesting.

THE BIOPHYSICAL MECHANISM OF ZEAXANTHIN/ΔpH-DEPENDENT ENERGY DISSIPATION WITHIN PSII IS UNRESOLVED

As already mentioned, there is compelling evidence that the presence of zeaxanthin within the PSII LHC ensemble and the generation of a large ΔpH across the thylakoid membrane (i.e. very low lumen pH) are simultaneously required to engage photoprotective thermal energy dissipation. One attractive proposal for the underlying biophysical basis for the reversible conversion between the high efficiency and photoprotective states centers on a lowered calculated energy of the xanthophyll excited state accompanying the conversion of violaxanthin to zeaxanthin (23). Thus the formation of zeaxanthin was envisioned to introduce a new, energetically favorable pathway that dramatically promoted thermal dissipation of excited chlorophyll molecules in the LHCII ensemble. However, very recently two different experimental procedures were devised to directly measure the energy levels of the previously inaccessible S_1 states of highly conjugated carotenoids (6, 18). These studies convincingly illustrated that the energy gap between the S_1 states of violaxanthin and zeaxanthin is too small to account for their differential quenching capabilities. A second proposal for the quenching mechanism arose from evidence that ΔpH-dependent accumulation of zeaxanthin results in the reversible oligomerization of LHCII (9). Aggregation was suggested to cause changes in orientation among the pigments bound to LHCII proteins, allowing pigment interaction leading to concentration quenching of chlorophyll excited states (i.e. increase in the thermal dissipation of absorbed light energy). In this proposal the xanthophyll cycle has an indirect role in thermal dissipation by mediating a critical conformational change within the PSII antenna.

Although the energy gap between the S_1 states of violaxanthin and zeaxanthin is now known to be only about one-half as large as previously thought, it is nevertheless true that direct quenching could contribute and thus may partner with changes in LHCII aggregation during the thermal dissipation process. Most importantly, this is a highly active area of research currently being explored from several different directions that point to exciting and perhaps surprising discoveries on the horizon.

WHAT HAPPENS IN PSI WHEN A LARGE PROPORTION OF THE LIGHT ENERGY ABSORBED BY PSII IS DISSIPATED AS HEAT?

Rarely discussed in the primary literature or in reviews on photoprotection in plants is the participation of PSI in thermal dissipation of excess absorbed light energy. At low irradiance levels when photosynthetic membranes are in the high-efficiency state (Fig. 1), leaves demonstrate an efficiency (i.e. quantum yield) for CO_2 reduction that is close to the theoretical maximum (13). This exceptionally high efficiency is possible only because the amount of light absorbed by the antenna serving the two photosystems is closely balanced. Thus it is inescapable that at high irradiance levels when PSII photoprotective thermal dissipation is engaged, PSI will be absorbing many more photons than it is receiving electrons from PSII. Cyclic electron flow around PSI may utilize some of this excess, but the capacity of this pathway is modest in comparison to the excess photon load when zeaxanthin/ΔpH-dependent energy dissipation is fully engaged in PSII.

Energy dissipation in PSI is much less studied than for PSII, but it is a reasonable notion that the photochemical yield in PSI is indirectly regulated by the photochemical yield in PSII. The central basis for this belief is that the oxidized primary donor of PSI, P_{700}^+, is a strong quencher of excited states in the PSI antenna and can accumulate when PSI photochemistry outpaces PSII. Although the photophysical mechanism of this quenching of chlorophyll excited states remains a matter of debate, it does provide a reasonable means to balance PSI light energy utilization via zeaxanthin/ΔpH-dependent energy dissipation in PS II. Thus, when PSI absorbs more light quanta than it receives electrons from PSII, P_{700} becomes oxidized and stays oxidized until an electron comes along from PSII. In this way, as depicted in Figure 1, thermal energy dissipation in PSI by P_{700}^+ quenching tracks the ΔpH-dependent regulation of PSII thermal energy dissipation (17).

LESSONS AND PROSPECTS

Although photodamage has been documented in crops grown outside of their ancestral geographic range, the vast majority of plants in native habitats and even most crops under cultivation deal successfully with excess light avoiding photodamage even under daunting environmental challenges. Photoprotection is a complex process that includes an array of alternative electron acceptors to utilize excess absorbed light when CO_2 is limiting, intricate pathways to detoxify photosynthetically produced reactive molecules, as well as a variety of repair processes to prevent the accumulation of photodamage. However, the regulated thermal dissipation of absorbed light is without question the keystone of photoprotection. There is a great deal of importance that is not yet understood about the mechanism and regulation of thermal dissipation, but the recent emergence of molecular genetic approaches portend rapid and exciting progress (14).

Emerging directly from these recent discoveries on regulated thermal dissipation is a current view of the

regulation of leaf photosynthesis as a balancing act in which photoprotection is traded for photosynthetic efficiency (16). It appears that evolution has refined the photosynthetic apparatus with an emphasis on high efficiency at limiting light with regulatory features to ensure that high intensities can be endured without the accumulation of photodamage. Although this view is admittedly an oversimplification, it is almost certainly true that when irradiances are high (e.g. mid-day at the top of the canopy) factors such as maintenance of water status take physiological precedence over maximizing photosynthesis. Although the trade-off between efficiency and photoprotection is clear, from an agricultural prospective it is less apparent how well the dynamic range of the trade-off is suited for agricultural environments and productivity goals. It seems possible, even likely, that forfeiture of photosynthetic efficiency may, under some circumstances, exceed that required to prevent photodamage thus reducing photosynthetic productivity more than necessary. Genetic variation in the ability of crop plant varieties to maintain photosynthetic efficiency at somewhat higher irradiances (i.e. higher ΔpH values) may prove to be an important factor in the search for improved photosynthetic productivity of crops.

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