Protection of the photosynthetic apparatus against damage by excessive illumination in homoiohydric leaves and poikilohydric mosses and lichens

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Abstract

Experimental work on the control of photosystem II in the photosynthetic apparatus of higher plants, mosses and lichens is reviewed on a background of current literature. Transmembrane proton transport during photoassimilatory and photorespiratory electron flows is considered insufficient for producing the intrathylakoid acidification necessary for control of photosystem II activity under excessive illumination. Oxygen reduction during the Mehler reaction is slow. Together with associated reactions (the water–water cycle), it poises the electron transport chain for coupled cyclic electron transport rather than acting as an efficient electron sink. Coupled electron transport not accompanied by ATP consumption in associated reactions provides the additional thylakoid acidification needed for the binding of zeaxanthin to a chlorophyll-containing thylakoid protein. This results in the formation of energy-dissipating traps in the antennae of photosystem II. Competition for energy capture decreases the activity of photosystem II. In hydrated mosses and lichens, but not in leaves of higher plants, protein protonation and zeaxanthin availability are fully sufficient for effective energy dissipation even when photosystem II reaction centres are open. In leaves, an additional light reaction is required, and energy dissipation occurs not only in the antennae but also in reaction centres. Loss of chlorophyll fluorescence during the drying of predarkened poikilohydric mosses and lichens indicates energy dissipation in the dry state which is unrelated to protonation and zeaxanthin availability. Excitation of photosystem II by sunlight is not destructive in these dry organisms, whereas photosystem II activity of dried leaves is rapidly lost under strong illumination.

Key words: Chlorophyll fluorescence, photoinactivation, photosystem II, proton transport, reaction centres, zeaxanthin.

Introduction

In photosynthesis, reaction centres of two photosystems convert photon energy into redox energy. The special pair of chlorophyll P_{680} in the reaction centre of photosystem II is photo-oxidized at a quantum efficiency close to 1. According to the structure of photosystem II reaction centres (Kurreck et al., 1997; Zouni et al., 2001), P_{680} is surrounded by four chlorophylls (Chl) and two pheophytins. An electron from the excited state (delocalized exciton) is separated from the hole located on P_{680} and transferred via Chl_{D1} to the nearby tetapyrrolic system of pheophytin_{D1}. Charge separation is stabilized by further electron transfer to a bound quinone termed Q_{A}. Photosystem II is a highly effective light-driven electron pump. As an extremely strong oxidant, P_{680} oxidizes...
water via a protein complex which includes a tyrosine residue and a manganese cluster. The electrons liberated during water oxidation travel, via photosystems II and I, to physiological acceptors such as CO₂.

The very efficiency of these light-driven electron transfer processes poses considerable problems for the stability of the photosynthetic apparatus. In contrast to higher plants, lichens and mosses are capable of tolerating full dehydration. What is the fate of the oxidant P₆₈₀ which is created in the reaction centre of photosystem II when water is unavailable as reductant? What happens to fully hydrated photosynthetic systems, when photon fluxes are high and physiological electron acceptors scarce so that excessive reduction of electron carriers stifles electron transfer? As oxygen is still abundantly available as a potential electron acceptor even when the leaves of higher plants close their stomata, restricting entry of CO₂, or when CO₂ diffusion is slowed down in lichens in rainy weather by films of water on the thallus surface (Lange et al., 2001), it could, in principle, serve as an alternative electron acceptor. However, the univalent reduction of oxygen in the Mehler reaction, when CO₂ is unavailable, would result in the production of reactive oxygen species such as superoxide and hydrogen peroxide which must be detoxified. It has been proposed that electron transfer to oxygen is an effective means to prevent excessive reduction of the electron transport chain (Schreiber and Neubauer, 1990; Osmond and Grace, 1995; Biehler and Fock, 1996; Asada, 1999). However, this view is not uncontested. The reduction of oxygen to the superoxide radical by photosystem I appears to be slow in many organisms (Wu et al., 1990; Heber et al., 1995; Ruuska et al., 2000; Cornic et al., 2000; Badger et al., 2000; Clarke and Johnson, 2001).

Photosystem II is known to be damaged when strong illumination causes either excessive reduction on the acceptor side or oxidation on the donor side (Anderson and Barber, 1996). Nevertheless, protective mechanisms exist. As early as 1957, Saposhnikov observed light-dependent carotenoid conversions. An action spectrum of the de-epoxidation of violaxanthin to zeaxanthin was published years later (Saposhnikov et al., 1966). This reaction requires a low intrathylakoid pH (Hager, 1969; Pfündel and Dilley, 1993) and is reversed in low light or darkness. In 1987, Barbara Demmig proposed that zeaxanthin is involved in the photoprotection of photosystem II (Demmig-Adams, 1990; Demmig-Adams and Adams, 1992). This xanthophyll appears to bind to the PsbS gene product, an intrinsic chlorophyll-containing protein in the antennae of photosystem II (Li et al., 2000). However, zeaxanthin is not sufficient for photoprotection. Another requirement is the protonation of a thylakoid protein (Horton et al., 1996; Gilmore and Govindjee, 1999) which presumably is identical with the PsbS gene product.

There is the question of how the intrathylakoid proton concentration is controlled in vivo, and which electron transport reactions contribute to it. During transport of one electron from water to NADP (and from there to CO₂), the proton/electron ratio is 3 (Hope et al., 1985; Ivanov et al., 1985; Rich, 1991; Kobayashi and Heber, 1995). CO₂ is reduced to the sugar level by 4 electrons. Therefore, the proton/CO₂ ratio is 12. Efflux of 12 protons through the ATPase of the thylakoid membrane permits the synthesis of 3 ATP (Rumberg et al., 1990; Kobayashi et al., 1995; Haraux and de Kouchkovsky, 1998). Recent information actually suggests that 14 protons rather than 12 are needed for the synthesis of 3 ATP (Seelert et al., 2000). The reduction of 1 CO₂ consumes 3 ATP. These relationships show that at least as many protons leave the thylakoids as are transported into the thylakoids during electron transport to CO₂. Clearly, proton pressure caused by electron transfer from water to CO₂ is unlikely to produce an intrathylakoid pH low enough for protein protonation especially as protonation would be expected to lead to decreased carbon assimilation owing to competition between assimilation and protonation-dependent energy dissipation.

Another important question is whether protein protonation and zeaxanthin are both necessary and sufficient to produce an energy trap that can compete efficiently with the reaction centres for energy capture, or whether protein protonation could do the job alone or in combination with another light-regulated reaction (Horton et al., 1996). Are there conditions where neither protonation nor zeaxanthin are required for the efficient transformation of the energy of absorbed light into heat? Some of the questions posed above will be considered briefly here.

**Transthalakoid proton gradient in relation to thermal dissipation of light energy in leaves**

Information on the magnitude of the transthylakoid proton gradient has been obtained for isolated thylakoids and chloroplasts mainly using the distribution of the fluorescent dye 9-aminoacridine as a criterion (Haraux and de Kouchkovsky, 1980; Vu Van and Rumberg, 1986). Values approaching and even exceeding ΔpH = 3 were often found but appear to give little information on the situation of a photosynthesizing leaf where proton deposition into the thylakoids and proton efflux from the thylakoids during ATP synthesis occur simultaneously. Since permeability barriers prevent the use of 9-aminoacridine for ΔpH measurements in leaves, another approach had to be sought. As the relationship between the rate of ATP synthesis and the magnitude of the proton motive force (pmf) is known (Junesch and Gräber, 1987; Possmeyer and Gräber, 1994) and the contribution of the membrane potential ΔΨ to the
two components of pmf, $\Delta pH$ and $\Delta \Psi$, is small, $\Delta pH$ can be calculated from carbon assimilation. CO$_2$ uptake was measured in leaves as a function of photon flux in the presence of 1% oxygen to prevent photorespiratory ATP turnover (Schönknecht et al., 1995). ATP/CO$_2$ is close to 3 in this situation. Simultaneously, both photochemical and non-photochemical quenching of chlorophyll fluorescence were measured and expressed in the form of the quenching coefficients $q_P$ and $q_N$ (for calculating these coefficients from fluorescence measurements see van Kooten and Snel, 1990). Because photochemistry, fluorescence and the dissipation of light energy in the form of heat are competitive, fluorescence measurements can give valuable information on light use by the photosynthetic apparatus (Schreiber et al., 1986; van Kooten and Snel, 1991; Krause and Weis, 1991). The fluorescence and gas exchange data of Schönknecht et al. show that light was used efficiently for carbon reduction already at $\Delta pH$ values close to 2 (Schönknecht et al., 1995). The indicator of the efficiency of photochemical light use, $q_P$, was close to 1 up to $\Delta pH$ values of about 2.4. It declined steeply with increasing $\Delta pH$. At $\Delta pH$ 2.6, $q_P$ had decreased to about 0.5. As expected, values for the indicator of energy dissipation $q_N$ were low up to $\Delta pH$ values of about 2.4. They increased steeply at higher $\Delta pH$ values and were not far from 0.8 at $\Delta pH$ 2.6. Importantly, the relationship between energy dissipation and $\Delta pH$ deviated strongly from what would be expected if energy dissipation is governed by the simple protonation of an amino acid residue of a protein. Formally, the steepness of the increase in energy dissipation with increasing $\Delta pH$ suggested hexa-co-operativity of proton action. According to very recent information, apparent hexa-co-operativity may result from the interaction of zeaxanthin with a protonated thylakoid protein which forms energy-dissipating exciton traps in the antennae of photosystem II (Bukhov et al., 2001a).

Which reactions contribute to the transthylakoid proton gradient in leaves?

Major electron transport pathways in higher plants and algae are those connected to Calvin cycle activity. Depending on ratios of CO$_2$ to O$_2$ at binding sites of ribulose bisphosphate carboxylase, ribulose bisphosphate is either carboxylated or oxygenated. In both cases, 3-phosphoglycerate is formed. After its phosphorylation by ATP, the final electron acceptor 1,3-bisphosphoglycerate is reduced by NADPH. Reduction of NADP to NADPH requires 2 electrons. The ATP/NADPH ratio is close to 1.5 not only in assimilatory carbohydrate production but also in photorespiratory carbohydrate oxidation. As outlined above, proton deposition into the thylakoids and proton outflow during assimilatory and photorespiratory electron transport reactions are therefore almost balanced. A proton gradient large enough to support much zeaxanthin formation and protein protonation cannot be formed.

The situation is different for photosynthetic nitrogen or sulphur assimilation and for oxygen reduction in the Mehler reaction. In these cases, coupled electron flow to NO$_3^-$, sulphate or oxygen is not, or not directly, accompanied by ATP consumption. In consequence, proton deposition during electron transport is not balanced by proton outflow. It can contribute to increased acidification of the thylakoid interior. However, although nitrogen assimilation (which is linked to carbon assimilation) contributes significantly to total electron flow in expanding leaves, nitrate reduction has never been observed to decrease electron flow in photosynthesizing leaves, although substrate amounts of nitrate have been shown to control electron flow in isolated chloroplasts via the transthylakoid proton gradient (Kobayashi et al., 1979). The absence of control of electron flow in leaves which are active in nitrogen assimilation shows that no extra proton deposition occurs which would lead to the zeaxanthin formation and protein protonation necessary for energy dissipation.

Electron flow to sulphate is much slower than electron flow to nitrite in photosynthesizing leaves. It cannot contribute appreciably to thylakoid acidification.

A more formidable candidate for an appreciable role in controlled energy dissipation is oxygen in the Mehler reaction. Its reduction is accompanied by coupled electron flow (Schreiber and Neubauer, 1990). However, thylakoid acidification in oxygen-reducing intact chloroplasts is larger than can be accounted for by the proton deposition during linear electron flow to oxygen (Kobayashi and Heber, 1994). Apparently, oxygen reduction and cyclic electron flow occur simultaneously. In recent experiments with barley leaves, Clarke and Johnson failed to obtain evidence for the control of photosystem II that could be attributed to oxygen reduction in the Mehler reaction (Clarke and Johnson, 2001). At low temperatures, the Mehler reaction actually appeared to be absent. Cyclic electron transport was considered to be responsible for producing the extra thylakoid acidification that leads to the effective dissipation of excess excitation energy as heat.

NADP is much preferred to oxygen as an electron acceptor in intact chloroplasts. Reduced NADP donates its electrons not only to 1,3-bisphosphoglycerate in assimilation and photorespiration, but also to oxaloacetate which is reduced to malate in isolated chloroplasts (Scheibe, 1990). Whereas bisphosphoglycerate reduction is not accompanied by coupled cyclic electron transport, the extent of thylakoid acidification during oxaloacetate reduction indicates the simultaneous occurrence of linear and cyclic electron transport (Ivanov et al., 1998). The
enzyme reducing oxaloacetate is regulated by light. It is active only at an increased NADPH/NADP ratio which permits the diversion of electrons into the cyclic pathway. A low NADPH/NADP ratio during bisphosphoglycerate reduction directs all electrons towards bisphosphoglycerate.

Nitrite is reduced in intact chloroplasts by reduced ferredoxin, not by NADPH. With nitrite as electron acceptor, thylakoid acidification is derived from linear electron transport only (Kobayashi and Heber, 1994; Ivanov et al., 1998). Apparently, rapid oxidation of reduced ferredoxin ensures the exclusive occurrence of linear electron transport as long as nitrite reduction does not approach saturation. Like a low NADPH/NADP ratio, rapid oxidation of reduced ferredoxin prevents the overflow of electrons into the cyclic pathway.

**Significance of oxygen reduction in leaves**

In the classical experiments of DI Arnon with isolated thylakoids, proper redox poising decided on the occurrence of cyclic photophosphorylation (Arnon and Chain, 1979). With an effective electron acceptor present and largely oxidized electron carriers in the electron transport chain, cyclic electron flow is suppressed in favour of linear electron flow. It is also suppressed when electron carriers between photosystem II and photosystem I are largely reduced and therefore incapable of accepting electrons from the cyclic pathway (Ziem-Hanck and Heber, 1980). Thus, the activity of photosystem II controls photosystem I activity.

The question of whether photosystem I can, in turn, control photosystem II (Heber and Walker, 1992) was answered when it was observed that in photosynthesizing leaves stomatal closure, which restricts the accessibility of CO₂ but not of oxygen (the oxygen/CO₂ ratio of air is 620), shifts the quantum efficiencies of electron flow through the photosystems from a 1/1 ratio to ratios indicating faster electron flow through photosystem I than through photosystem II (Gerst et al., 1995). More direct information was obtained when dark-adapted leaves (to inactivate light-regulated enzymes of the Calvin cycle) were illuminated with far-red light which is weakly absorbed by photosystem I and almost not by photosystem II. As long as the Calvin cycle was not activated (i.e. as long as linear electron flow remained inactive) the quantum yield of photosystem II reactions was much decreased even in the presence of air levels of oxygen indicating either transient down-regulation of photosystem II by far-red light, or far-red-dependent formation of an energy trap which can compete efficiently with photosystem II for light capture (Cornic et al., 2000). Importantly, optical measurements at 505 nm indicated that zeaxanthin was formed under far-red illumination as long as linear electron transport was slow. Zeaxanthin synthesis ceased when the acceptor side of photosystem I was opened for linear flow. The observations show that far-red light supported coupled cyclic electron transport at rates sufficient to decrease the intrathylakoid pH, not only for zeaxanthin synthesis but also for the protonation reaction that permits dissipation rather than conservation of light energy. Energy dissipation was replaced by energy conservation as soon as electrons, drained from the electron transport chain, were permitted to travel to external acceptors.

It must be emphasized that coupled linear electron flow to oxygen in the Mehler reaction was not capable, under far-red light, of decreasing the intrathylakoid pH sufficiently for photosystem II control, whereas cyclic electron transport was effective. Oxygen acted as poising agent rather than as an efficient electron acceptor. When methylviologen was added as an intermediate electron acceptor on the path to oxygen, electron flow was accelerated and cyclic electron flow was suppressed. Even the accelerated linear flow failed to control photosystem II activity under far-red light. The observations show that the concept of redox poising which was explored by Arnon for thylakoids several decades ago is also valid for leaves. It assigns an important physiological role to photorespiratory carbohydrate oxidation (Wu et al., 1990; Heber et al., 1995; Kozaki and Takeba, 1996; Wiese and Heber, 1998). When stomata are closed under water stress, the linear electron transport permitted by the interplay of photorespiratory CO₂ release and refixation of the released CO₂ prevents the excessive reduction of electron carriers which would block coupled cyclic electron transport. Even though there is little doubt that univalent oxygen reduction in the Mehler reaction acting in concert with cyclic electron flow can control photosystem II activity under strong light (Schreiber and Neubauer, 1990; Kobayashi and Heber, 1994), oxygen reduction itself appears to be too slow in leaves (Badger et al., 2000; Clarke and Johnson, 2001) to prevent the increased reduction of electron carriers which leads to photodamage (Anderson and Barber, 1996). In intact chloroplasts reduction of the electron transport chain increased when cyclic electron transport was inhibited by antimycin A during electron transport to oxygen (Ivanov et al., 1998). Increased reduction increases sensitivity to photoinhibition.

**Is zeaxanthin indispensable for dissipation of excess light energy in leaves?**

Much information is available on light-dependent zeaxanthin formation and its role in the dissipation of excess light energy and a large body of the literature has been reviewed (Demmig-Adams and Adams, 1992;
Is zeaxanthin indispensable for dissipation of excess light energy in mosses?

Slow drying of a poikilohydric moss in the sun (as it normally occurs after nightly hydration) results in dramatic quenching of chlorophyll fluorescence. In the dry moss, photochemical energy use was no longer indicated by light-dependent increases in chlorophyll fluorescence. In the absence of measurable photochemistry, decreased fluorescence indicates increased energy dissipation. No loss of fluorescence was observed when leaves, after removing their epidermis to make drying comparable to that experienced by the moss, were dehydrated. Rehydration resulted in a rapid increase of fluorescence of the moss, whereas hydration of dried leaves actually decreased fluorescence (Heber et al., 2000). The capacity for photochemical energy conversion recovered rapidly in the moss during rehydration. Light in excess to that which can be used for photochemistry was effectively dissipated as shown by dramatically increased non-photochemical fluorescence quenching in strong light. In fact, excess light was not even needed for energy dissipation in hydrated Grimmia alpestris (Heber et al., 2000) or Rhytidiadelphus squarrosus (Bukhov et al., 2001a) which had been illuminated before darkening to accumulate some zeaxanthin. In the presence of zeaxanthin, but not in its absence, protonation by high concentrations of CO₂ produced strong quenching even of so-called ‘dark’ fluorescence, i.e. the fluorescence elicited by red light at an intensity which is too low to produce easily measurable photochemistry. Such quenching is always an indication of effective zeaxanthin-dependent energy dissipation. No comparable quenching of ‘dark’ fluorescence has been observed in leaves (Bukhov et al. 2001b). However, when after prolonged darkening zeaxanthin had been reconverted to violaxanthin, protonation by CO₂ was no longer able to quench ‘dark’ fluorescence in the moss. The experiments demonstrated that in the hydrated moss (but not in leaves) protonation and zeaxanthin were fully sufficient for effective energy dissipation. In accordance with this, a comparison of model predictions with experimental data suggested that practically all energy dissipation occurred in the hydrated moss in the antennae and practically none in the reaction centres of photosystem II (Bukhov et al., 2001b). However, when zeaxanthin synthesis was suppressed by DTT, energy dissipation shifted to the reaction centres.

A comparison of non-photochemical fluorescence quenching (i.e. of energy dissipation) with zeaxanthin contents in the moss revealed that less than three molecules of zeaxanthin were sufficient to compete with a reaction centre of photosystem II for energy capture on equal terms provided the intrathylakoid pH was low enough for efficient protein protonation (Bukhov et al., 2001a).
In leaves, mathematical analysis had suggested hexa-co-operativity of proton action on energy dissipation (Schönknecht et al., 1995). In the hydrated moss, almost no ‘dark’ energy dissipation was produced by high concentrations of the protonating agent CO₂ as long as zeaxanthin was absent, but protonation became highly effective when zeaxanthin was present (Heber et al., 2000; Bukhov et al., 2001a). This suggests that what had been formally described as hexa-co-operativity of proton action was in fact the result of binding of zeaxanthin to a protonated chlorophyll-containing protein, perhaps the PsbS gene product (Li et al., 2000). This converts this chlorophyll-containing protein into an efficient energy trap (Gilmore and Govindjee, 1999).

Is zeaxanthin indispensable for dissipation of excess light energy in lichens?

The answer to this simple question depends on whether a lichen is associated with green algae or cyanobacteria. The latter do not possess a violaxanthin/zeaxanthin cycle. Obviously, in their case, zeaxanthin cannot be an important factor in energy dissipation. When lichens, whether ‘green’ or ‘bluegreen’, dry in the sun after nightly hydration, chlorophyll fluorescence decreases strongly and light-dependent charge separation in the reaction centre of photosystem II is much decreased. Photosystem I may remain fully active as observed in dry Xanthoria elegans which is associated with green algae (Heber et al., 2000) or in dry Peltigera rufescens which contains cyanobacteria. Oxidized P₇₀₀ in the reaction centre of photosystem I is known to be very effective in dissipating absorbed light energy as heat. In other air-dried lichens with green algae such as Parmelia sulcata and Hypogymnia physodes, P₇₀₀ oxidation was not observed on illumination, perhaps because the pigment was already oxidized. Rehydration of the lichens resulted in all cases in rapidly increased fluorescence and the recovery not only of photosystem I but also of sensitive photosystem II photochemistry. Recovery was very fast in the ‘green’ lichens Parmelia sulcata and Hypogymnia physodes and much slower in ‘bluegreen’ Peltigera rufescens. In general, the response of chlorophyll fluorescence of lichens with green algae was similar to that of the poikilohydric moss Rhytidiothecium squarrosum. This was true not only for dehydration and rehydration but also for DTT treatment which inhibited non-radiative energy dissipation, and for the addition of high concentrations of CO₂ to darkened lichens which decreased ‘dark’ fluorescence only after previous illumination, but not after prolonged darkening during which photo-accumulated zeaxanthin is known to be reconverted into violaxanthin (Heber et al., 2000). In illuminated zeaxanthin-containing lichens coupled cyclic electron transport appeared to be a major source of thylakoid acidification, but addition of high concentrations of CO₂ further increased energy dissipation. Importantly, high concentrations of CO₂ were ineffective to quench fluorescence in ‘bluegreen’ Peltigera rufescens (unpublished observations). It thus appears that for hydrated lichens with green algae zeaxanthin and photosystem II are sufficient for efficient non-radiative energy dissipation in traps which are formed in the antennae of photosystem II. However, the loss of chlorophyll fluorescence during drying does not appear to require the presence of zeaxanthin because it was similar in light- and dark-adapted ‘green’ lichens and occurred also in ‘bluegreen’ Peltigera.

Outlook

Many questions remain to be answered in further work. Why is the combination of zeaxanthin and a low intrathylakoid pH sufficient in some hydrated mosses and ‘green’ lichens, but not in higher plants, to dissipate the energy even of extremely low light in the antennae of photosystem II rather than to use it for energy conservation in open reaction centres? Which reaction is responsible in higher plants for the additional light requirement of effective energy dissipation in the antennae? What is necessary to convert the reaction centre of photosystem II from an energy-conserving into an energy dissipating centre? What is responsible for the stabilization of the photosynthetic apparatus of poikilohydric mosses and lichens, either associated with green algae or cyanobacteria, to tolerate dehydration under sunlight? Stabilization finds expression in loss of chlorophyll fluorescence and loss of easily measurable charge separation in photosystem II. What is the difference to leaves of higher plants which do not manage to quench chlorophyll fluorescence during drying, and which maintain effective charge separation in the dry state giving rise to photochemical reactions which damage the reaction centres? Answers to these questions will require a careful examination of the photochemistry of reaction centres of different photosynthetic organisms at different water potentials down to the water potential of desert air on a sunny summer day.

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References

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