THE ROYAL SOCIETY

*Published online* 2 October 2002

# Structural and functional dynamics of plant photosystem II

**Jan M. Anderson*** **and W. S. Chow**

*Photobioenergetics, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra ACT 2601, Australia*

Given the unique problem of the extremely high potential of the oxidant $P_{680}^{+}$ that is required to oxidize water to oxygen, the photoinactivation of photosystem II *in vivo* is inevitable, despite many photoprotective strategies. There is, however, a robustness of photosystem II, which depends partly on the highly dynamic compositional and structural heterogeneity of the cycle between functional and non-functional photosystem II complexes in response to light level. This coordinated regulation involves photon usage (energy utilization in photochemistry) and excess energy dissipation as heat, photoprotection by many molecular strategies, photoinactivation followed by photon damage and ultimately the D1 protein dynamics involved in the photosystem II repair cycle. Compelling, though indirect evidence suggests that the radical pair $P_{680}^{+}Pheo^{-}$ in functional PSII should be protected from oxygen. By analogy to the tentative oxygen channel of cytochrome *c* oxidase, oxygen may be liberated from the two water molecules bound to the catalytic site of the Mn cluster, via a specific pathway to the membrane surface. The function of the proposed oxygen pathway is to prevent $O_2$ from having direct access to $P_{680}^{+}Pheo^{-}$ and prevent the generation of singlet oxygen via the triplet-$P_{680}$ state in functional photosytem IIs. Only when the, as yet unidentified, potential trigger with a fateful first oxidative step destroys oxygen evolution, will the ensuing cascade of structural perturbations of photosystem II destroy the proposed oxygen, water and proton pathways. Then oxygen has direct access to $P_{680}^{+}Pheo^{-}$, singlet oxygen will be produced and may successively oxidize specific amino acids of the phosphorylated D1 protein of photosystem II dimers that are confined to appressed granal domains, thereby targeting D1 protein for eventual degradation and replacement in non-appressed thylakoid domains.

**Keywords:** oxygen pathway; $P_{680}^{+}$; photosystem II; photoinactivation; photosynthesis; singlet oxygen

## 1. INTRODUCTION

The remarkable robustness of higher-plant PSII *in vivo* depends on the complementary logic inherent in this dynamic molecular machine, which is highly regulated by light in both its functional and non-functional state. This regulation involves photon use (energy utilization for electron transport and proton transfer) versus energy dissipation, photon protection (many photoprotective strategies) versus photoinactivation and D1 protein repair. Given the unique problem of the extremely high potential of PSII that is needed to oxidize water to oxygen, protons and electrons, its photoinactivation is inevitable, despite many photoprotective strategies. PSII has a limited functional life as PSII dimers in the appressed granal thylakoids. This leads to a highly dynamic compositional and structural heterogeneity of the cycle between functional and non-functional PSII complexes in response to light level. Ultimately, D1 protein dynamics are involved in the PSII repair cycle: unlike Humpty Dumpty, PSII can be put together again with relatively little energy cost.

PSII, a multi-subunit pigment–protein complex consisting of at least 25 different proteins located in thylakoid membranes, catalyses the light-induced splitting of water to molecular oxygen and reducing equivalents ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$). However, to oxidize extremely stable water, the strongest biological oxidant known, $P_{680}^{+}$, is generated, making PSII particularly vulnerable to light and oxidative stress. At the heart of PSII, the reaction centre D1/D2 protein heterodimer binds all of the redox factors necessary for stable light-induced charge separation across the thylakoid membrane (Diner & Babcock 1996). On illumination, $P_{680}$, a special Chl *a* molecule or multimeric Chl *a* molecules absorbing at 680 nm (Dekker & Van Grondelle 2000), initially forms the excited singlet state and donates an energized electron, within a few picoseconds, to a Pheo molecule to form the primary radical pair, $P_{680}^{+}Pheo^{-}$. Within 300 ps, $Pheo^{-}$ passes an electron to the primary plastoquinone electron acceptor, $Q_A$. $P_{680}^{+}$ is reduced within nano- to microseconds by a redox-active tyrosine residue $Y_Z$ (Tyr 161-D1 protein). $Y_Z^{ox}$ is successively reduced by the tetra-manganese cluster that stores the oxidation equivalents required to oxidize water to oxygen. The oxygen-evolving core complex comprises other proteins, closely associated with the D1/D2 heterodimer. These are the Chl *a* proteins, CP43 and CP47, α- and β-subunits of cytochrome *b*559, several small membrane-spanning helical proteins, a cluster of four manganese atoms probably all attached to D1 protein and the 33 kDa,

*Author for correspondence (anderson@rsbs.anu.edu.au).

One contribution of 21 to a Discussion Meeting Issue 'Photosystem II: molecular structure and function'.

23 kDa and 17 kDa extrinsic lumenal proteins (Barber 1998). In higher plants, and some green algae, the central core is flanked by a family of chlorophyll *a*/*b* proteins, monomeric CP29, CP26 and CP24, and the outer antenna trimeric LHCII that regulate light-harvesting (Horton *et al.* 1996).

Paradoxically, as a dynamic molecular machine, PSII has a limited functional life. The extremely high oxidizing potential of $P^{+}_{680}$ (estimated as greater than 1.12 V; Klimov *et al.* 1979) means that PSII function is always at risk. The inevitable photoinactivation of PSII, discussed below (see § 3) is matched by several interacting photoprotective strategies that help to protect PSII from excess photons. Recovery from photoinactivation via D1 protein degradation and *de novo* D1 protein synthesis is the most vital strategy (Prásil *et al.* 1992; Aro *et al.* 1993). The turnover of D1 protein in the PSII reaction centre increases linearly with irradiance to beyond light saturation (Anderson & Aro 1994), but significantly, the repair process saturates at low light (Anderson 1999). The rate of repair of PSII is fast enough to prevent net photoinactivation under normal irradiance. However, in higher plants under sustained high light, particularly combined with other environmental stress, photoinactivated PSII reaction centres still possessing damaged D1 protein accumulate in stacked granal domains: here, PSII is protected from disassembly since D1 protein degradation is prevented. When normal irradiance is received again, these PSII centres are repaired in unstacked thylakoid membranes via the coordinated processes of D1 protein degradation and *de novo* synthesis.

Part of the robustness of plant PSII depends on the multiple, light-regulated photoprotective strategies that have evolved to balance light utilization versus light dissipation. These include physiological responses that decrease incident light on PSII *in situ* by leaf and chloroplast movement, waxy cuticles, anthocyanin content and so on. At the molecular level, under light saturation, the rate of excitation of the photosynthetic apparatus greatly exceeds the utilization of excitation energy by electron transport. The excess excitation is mostly converted to heat (NPQ), which results from the combined effects of the increase in transthylakoid $\Delta$pH and the de-epoxidation of the carotenoid, violaxanthin to form zeaxanthin (Horton *et al.* 1996).

Dynamic photosynthetic acclimation also modulates energy utilization via molecular mechanisms, such as state transitions and cyclic electron transport around each photosystem. In addition, photosynthetic and metabolic acclimation lead to changes in composition that, in turn, influence structure and function (Anderson *et al.* 1997). For example, plants acclimated to high light have more PSII units, each with smaller light-harvesting antennae relative to PSI, together with enhanced capacities of electron transport, ATP synthesis and carbon assimilation coupled to enhanced NPQ to mitigate against the photoinactivation of PSII. Conversely, shade and low-light plants acclimated to low light have fewer PSII units with much larger light-harvesting antennae to maximize light absorption relative to PSI: they have lower capacities for maximum photosynthesis, carbon assimilation and NPQ, and are readily photoinhibited when shifted to high light (Anderson *et al.* 1997).

These photoprotective responses, which regulate and optimize PSII function, together with oxygen scavenging systems, are integrated into a dynamic time continuum. Nevertheless, there is a small but finite probability that PSII is inactivated by light and hence unable to evolve oxygen. The rate of repair of PSII by degradation of damaged D1 protein and its *de novo* synthesis is usually fast enough to prevent net photoinactivation. When repair is retarded, or photoinactivation is accelerated under high light, particularly combined with another environmental stress such as chilling, a loss of PSII function occurs. Any net loss of PSII function under limiting light will decrease photosynthetic efficiency: when *ca*. 40% of PSII is inactivated, the maximum photosynthetic rate will then be limited in high light (Chow 2001). Huge dynamic compositional and structural light-regulated changes are needed to restore PSII function. Non-functional phosphorylated PSII dimers are confined to appressed grana domains prior to the migration of PSII monomers out to non-appressed domains (Baena-González *et al.* 1999; Rintamäki *et al.* 1999). Here, PSII function is restored when the damaged D1 protein is degraded and simultaneously replaced by new D1 protein synthesized on chloroplast ribosomes, processed and reassembled with D2 protein and CP43 (Zhang *et al.* 1999).

## 2. THE MOLECULAR ENVIRONMENT AROUND $P^{+}_{680}$

The unique feature of the PSII complex is the extremely high oxidizing potential of $P^{+}_{680}$ (greater than 1.12 V) (Klimov *et al.* 1979), in contrast to 0.4–0.6 V for all other oxidized primary donors of plant and bacterial reaction centres (Barber 1995). Clearly, the environment around $P^{+}_{680}$ must be unusual to prevent the oxidation of neighbouring chlorophylls or amino acids. The robustness of functional plant PSII depends partly on the structural integrity of the protein matrix surrounding $P_{680}$.

### (a) *The role of* $P^{+}_{680}$

In $P_{680}$, the two chlorophyll *a* molecules that are ligated to His 198 on D1 and D2 proteins are parallel and arranged perpendicular to the membrane plane, with an Mg–Mg distance of 10 Å (Zouni *et al.* 2001). With less overlap between the chlorin rings of $P_{680}$ compared with other photosynthetic primary donors, excitonic coupling is much weaker than in photosynthetic bacteria, but importantly, charge separation across the membrane is twice as efficient (Diner & Babcock 1996). The positive charge of $P^{+}_{680}$ may be shared between the four chlorophylls of the PSII reaction centre dimer (special pair and two accessory chlorophylls) due to a multimeric organization (Dekker & Van Grondelle (2000) and references therein). Mulkidjanian (1999) suggests that the high oxidizing potential of $P^{+}_{680}$ may be attributed to an electrostatic influence dominated mainly by Arg-181 of the D2 subunit and the experimentally demonstrated retarded protonic relaxation at the $P_{680}$ site.

This arrangement governs two unique features of energy transfer within PSII (figure 1). With weak excitonic coupling, $P_{680}$ is a 'shallow trap' with a small energy gradient from the many antenna chlorophyll *a* and *b* molecules to $P_{680}$. Energy transfer and trapping are in a reversible equilibrium that involves both the exciton and the primary

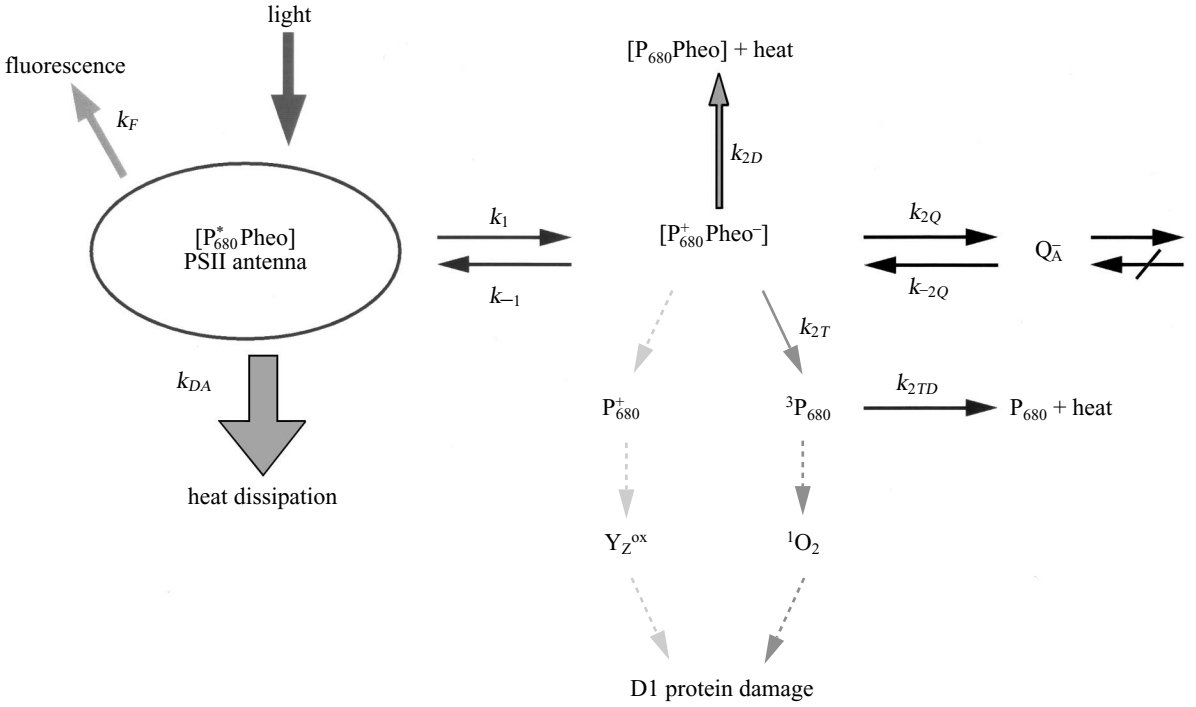


Figure 1. A schematic for energy transfer in PSII *in vivo* based on the exciton–radical pair equilibrium model of Schatz *et al.* (1988). Light absorbed by the PSII antenna will be dissipated by three reactions: (i) excitation trapping ($k_1$); (ii) fluorescence ($k_F$); (iii) non-radiative heat dissipation mainly by antenna ($k_{DA}$), but also by triplet $P_{680}$ quenching ($k_{2TD}$) and possibly reaction-centre quenching ($k_{2D}$). The primary radical pair will decay by four reactions: (i) charge recombination ($k_{-1}$); charge stabilization by electron transfer to $Q_A$ ($k_{2Q}$); (iii) non-radiative transfer to ground state ($k_{2D}$); spin-dephasing to the triplet state of the radical pair followed by formation of triplet $P_{680}$ ($k_{2T}$). The dotted pathways indicate possible oxidative damage to D1 protein by $P^+_{680}$, $Y_Z^{ox}$ or singlet oxygen generated by triplet $P_{680}$. (Redrawn from Anderson *et al.* (1998).)

radical pair, $P^+_{680}Pheo^-$ (Schatz *et al.* 1988). Stable charge separation takes place when an electron from reduced $Pheo^-$ is transferred to the primary electron acceptor, $Q_A$, and the exciton is dissipated. However, charge recombination between $P^+_{680}$ and $Pheo^-$ can result in a re-formation of the singlet-excited state of $P_{680}$ ($P^*_{680}$), and subsequent re-equilibration of excitation among the antenna chlorophylls and non-radiative dissipation may occur. Hence, an exciton may visit the reaction centre several times before being trapped by electron transport or dissipated from the antenna as heat. When electron transport is blocked and $Q_A^-$ can no longer transfer an electron to $Q_B$, charge recombination occurs between $P^+_{680}$ and $Pheo^-$ to yield an exciton in the antenna. This exciton–primary radical pair equilibrium, which operates in both functional and non-functional PSIIs, contributes greatly to the robustness of PSII (figure 1). Significantly, even in non-functional PSII, re-equilibration of excitation among antenna chlorophylls allows non-radiative dissipation.

A complication arises, however, since the radical pair occurs in both the singlet and triplet state. Charge recombination of the singlet state of the radical pair yields singlet-excited $P^*_{680}$, whereas the triplet state of the radical pair yields triplet $P_{680}$ ($^3P^*_{680}$). Triplet $P_{680}$ may return directly to the ground state or react with oxygen to yield highly reactive singlet oxygen ($^1O_2$).

$$^3P_{680} + {}^3O_2 \rightarrow P_{680} + {}^1O_2.$$

### (b) *The role of β-carotene in PSII reaction centre*

Carotenoids are essential components of all Chl-proteins since they protect chlorophyll from the potentially damaging effects of light and oxygen. Apart from general NPQ by the xanthophyll cycle, carotenoids have two essential photoprotective roles: quenching excitation energy from triplet chlorophyll and singlet oxygen (Sieffermann-Harms 1987).

(i) Triplet chlorophyll ($^3$Chl) is rapidly quenched by carotenoids by triplet–triplet energy transfer, followed by triplet carotenoid ($^3$Car) returning to the ground state with heat dissipation.

$$^3Chl + Car \rightarrow Chl + {}^3Car,$$

$$^3Car \rightarrow Car + heat.$$

(ii) Singlet oxygen may react with carotenoids to form $^3$Car, which decays to the ground state with heat dissipation.

$$^1O_2 + Car \rightarrow {}^3Car + {}^3O_2,$$

$$^3Car \rightarrow Car + heat.$$

In leaves, the faster reaction (i) is predominant (90–95%) (Sieffermann-Harms 1987). However, in functional PSII during repeated $P_{680}$ turnover, β-carotene is unable to quench $^3P^*_{680}$ by triplet–triplet exchange when isolated PSII reaction centres are subjected to very high light (Barber 1995; Telfer 2002). Presumably, the high potential of $P^+_{680}$ would oxidize β-carotene if it were close enough for direct carotenoid quenching (Barber 1995; Telfer 2002). While β-carotene may help to deactivate some of the $^1O_2$ generated by $^3P^*_{680}$ *in vitro* (Barber 1995), is the formation of singlet oxygen actually prevented in functional PSII *in vivo*?

## 3. PHOTOINACTIVATION OF PSII *IN VIVO* UNDER STEADY-STATE PHOTOSYNTHESIS

### (a) *The photoinactivation of PSII depends on the light dose*

In steady-state photosynthesis, the extent of photoinactivation of PSII shows reciprocity between the irradiance level and the duration of illumination. It depends on the total incident photon exposure (the product of irradiance and time of illumination) in isolated spinach thylakoids (Jones & Kok 1966), cyanobacteria and leaf discs of several plant species (see Anderson *et al.* 1998 for references). Consequently, PSII photoinactivation *in vivo* depends on the number of photons absorbed and not the rate of absorption *per se*. Being a light-dosage effect, it occurs under all irradiances. When a certain number of photons have been absorbed by the many PSIIs in a leaf, there is a finite probability of an individual PSII being inactivated. The quantum yield is low; typically, one PSII is photoinactivated per $10^7$ photons (Anderson *et al.* 1998; Chow 2001). This quantum yield is not unique, but varies with the size of light-harvesting antennae associated with PSII, the PSII/PSI stoichiometry, the state of acclimation of other thylakoid components and varying extents of multiple photoprotective strategies and environmental stress.

### (b) *Photoinactivation of PSII depends on the redox state of $Q_A^-$*

It was assumed that photoinactivation *in vivo* occurs due to excessive excitation pressure on PSII, with $Q_A$ being mainly reduced under sustained high light (Prásil *et al.* 1992; Aro *et al.* 1993). However, over-reduction of $Q_A$ is not a prerequisite since PSII function begins to be lost when some 40% of the PSII reaction centres are closed (*ca.* 40% of $Q_A^-$) (Öquist *et al.* 1992). Photoinactivation of PSII is a unique function of both sun and shade plants, which is inevitable as soon as *ca.* 40% of the PSII traps become closed. Further, PSII reaction centres have identical intrinsic susceptibilities to photoinactivation, irrespective of light acclimation during growth (Park *et al.* 1996*a*). This means that the modulation of resistance to photoinactivation is controlled by factors that determine the redox state of $Q_A$, by the flow of photons into $P_{680}$ (affected by antenna size, PSII/PSI stoichiometry and non-photochemical quenching) and the flow of electrons out from $Q_A^-$ (Öquist *et al.* 1992). Most leaves are able to balance energy consumed by PSII (measured as the fluorescence parameter, $1 - qP$) with energy dissipated by NPQ monitored by chlorophyll fluorescence (Park *et al.* 1996*c*). Significantly, the quotient $(1 - qP)$/NPQ is roughly constant over the entire irradiance range, although the actual value depends on growth light conditions (Park *et al.* 1996*c*). The robustness of PSII during steady-state photosynthesis under non-stressed conditions depends partly on the amazing ability of plants and algae to balance light supply with light consumption over the entire irradiance range.

### (c) *Potential trigger for the initial act of photoinactivation* in vivo *under steady-state photosynthesis*

In considering the robustness of PSII, it is important to define the primary cause of the inevitable photoinactivation *in vivo*. However, the identity of the initial trigger for photoinactivation under steady-state photosynthesis is not known. Arising mainly from *in vitro* studies, two mechanisms were proposed to target D1 protein for degradation, one linked to the donor side and the other to the acceptor side of PSII (Prásil *et al.*1992; Aro *et al.* 1993; Barber 1995). In the donor-side mechanism, the supply of electrons from water to $P_{680}$ does not match the removal of electrons from $Pheo^-$ and the enhanced cation radicals $P_{680}^+$ or $Tyr_Z^{ox}$, being very strong oxidants, could trigger photoinactivation, in the presence or absence of $O_2$. In the acceptor-side mechanism, high light leads to structural alteration at the acceptor side that blocks electron flow from $Q_A^-$ to $Q_B$, then $Q_A$ may be doubly reduced to $Q_AH_2$ and released from its binding site. The loss of $Q_A$ increases the probability of charge recombination of the primary radical pair, thereby facilitating the formation of triplet $P_{680}$ ($^3P_{680}$) that reacts with $O_2$ to generate highly toxic singlet oxygen ($^1O_2$).

Since PSII photoinactivation *in vivo* depends on the light dose and reciprocity is observed at all levels of irradiance from limiting to supersaturating light, the probability of activating the potential trigger should be independent of irradiance. Reciprocity implies that there should only be a single molecular mechanism for photoinactivation *in vivo* occurring under all light levels in steady-state photosynthesis (Anderson *et al.* 1998). In turn, this implies only a single potential trigger. From these considerations and other indirect evidence, $P_{680}^+$ has been proposed as a potential trigger (Anderson *et al.* 1998; Chow 2001). However, Oxborough & Baker (2000), when analysing fluorescence data, suggested rather that the formation of $^3P_{680}$ (through charge recombination rather than intersystem crossing), as well as double reduction of $Q_A$, are likely potential triggers. Recently, new candidates—$Mn^{3+}$ or $Mn^{4+}$ of the oxygen-evolving complex—have been postulated by Tyystjärvi *et al.* (2002), who suggest that the action spectrum of Jones & Kok (1966) implies that photoinhibition is actually a UV-light phenomenon. By contrast, Vass *et al.* (2002) maintain that different mechanisms are needed for visible and UV-light photoinhibition. Hence, neither the potential trigger for photoinactivation nor the actual number of molecular processes involved is yet established. It is important, however, to remember that following the initial photoinactivation of PSII, light continues to generate highly toxic species in non-$Q_B$-reducing PSII (due to the exciton–radical pair equilibrium), which will damage D1/D2 proteins and the bound redox factors.

## 4. THE OXYGEN ACCESSIBILITY HYPOTHESIS FOR PSII

Perhaps oxygen molecules are not freely accessible to $P_{680}$ in functional PSII (Anderson *et al.* 1998; Anderson 2001). The oxygen liberated from the two water molecules bound to the catalytic site in the manganese cluster will be directed out to the membrane surface by a specific pathway whose purpose is to prevent oxygen being accessible to the radical pair. The tightly packed hydrophobic protein domain around $P_{680}$ in active PSII may also shield $P_{680}$ from oxygen. If this is true, the generation of highly toxic singlet oxygen, via the $P_{680}$ triplet state, would be impossible in functional PSII *in vivo*. However, once PSII

is photoinactivated, singlet oxygen could be generated in non-$Q_B$-reducing PSIIs if the structural integrity of the proposed oxygen pathway is destroyed following the initial act of photoinactivation. Then oxygen could be directly accessible to $P^+_{680}Pheo^-$ formed by change recombination and $^3P_{680}$ could react with oxygen to form singlet oxygen.

## 5. EVIDENCE FOR THE LACK OF ACCESSIBILITY OF OXYGEN IN FUNCTIONAL PSII

### (a) *Accessibility of oxygen to $P^+_{680}Pheo^-$ in functional PSII*

Evidence for the lack of accessibility of oxygen to the primary radical pair in functional PSII comes from Hideg *et al.* (1998), who devised a novel method to detect singlet oxygen production in leaves. Singlet oxygen was generated from bean leaves only after some PSIIs were photoinactivated, and the rate of $^1O_2$ production was proportional to the number of non-functional PSIIs, although the actual amount of $^1O_2$ per $P_{680}$ could not be determined (Hideg *et al.* 1998). If the radical pair were accessible to oxygen in functional PSII, the generation of singlet oxygen should be proportional to the number of functional PSIIs. This implies that with structurally perturbed non-$Q_B$-reducing PSII, oxygen has access to $P_{680}$, but functional PSIIs are not initially photoinactivated by singlet oxygen. If only a single mechanism is required for the initial photoinactivation, as implied by reciprocity, then the initial trigger is unlikely to be singlet oxygen.

Since the photoinactivation of PSII proceeds rapidly at low photon exposure under nitrogen and the reciprocity law still holds, oxygen is not required unless it originates directly from water splitting (Park *et al.* 1996*c*; figure 2). This favours, but does not prove, a single molecular mechanism for the initial photoinactivation of PSII that does not require oxygen.

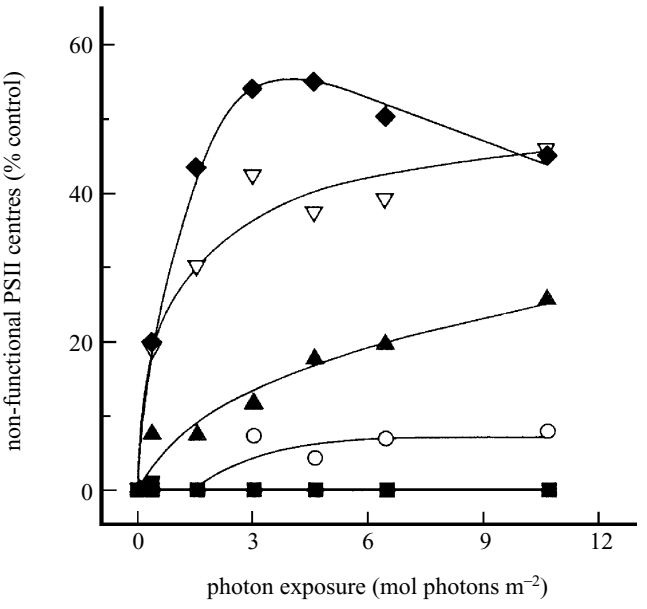


Figure 2. Photoinactivation of PSII in pea leaves as a function of photon exposure. The accumulation of non-functional PSII centres in pea leaves under nitrogen compared with control leaves in air and in the presence of inhibitors of photoprotection, nigericin (ΔpH), lincomycin (chloroplast protein synthesis) or dithiothreitol (DTT) (xanthophyll cycle) compared with untreated control leaves. Note that with increasing photon exposure, prevention of high ΔpH by nigericin increased the rate and extent of formation of non-functional PSII compared with prevention of the xanthophyll cycle (DTT). Key: filled diamonds, 100% $N_2$; open inverted triangles, inhibited ΔpH, D1 synthesis; filled triangles, inhibited ΔpH; open circles, inhibited V → A+Z; filled squares, control leaves. (Redrawn from Park *et al.* (1996*b*,*c*).)

### (b) *Accessibility of oxygen within other membrane protein complexes*

Limited accessibility of oxygen within other membrane protein complexes has been suggested. However, an oxygen pathway has only been postulated for cytochrome *c* oxidase following its high-resolution structure (see § 5b(iii)).

#### (i) *Photosystem I*

Since the generation of $^3P_{700}$ in isolated PSI was unaffected by oxygen, Setif *et al.* (1981) proposed that oxygen should be inaccessible to $P_{700}$. This screening of triplet $P_{700}$ from oxygen was suggested to be a functional prerequisite for PSI, in order to avoid scavenging of the low-potential reductants of oxygen. Such a proposal might now be tested by dynamic molecular simulations of oxygen diffusion within the high-resolution structure of PSI (Jordan *et al.* 2001).

#### (ii) *Trimeric light-harvesting complex*

Significantly, both oxygen and proton barriers have been demonstrated in the very hydrophobic light-harvesting Chl *a*/*b*-proteins that house most of the Chl *a*, Chl *b* and carotenoid molecules of plant PSII (Sieffermann-Harms & Angerhofer 1998). Isolated phototostable LHCII trimers have protected pigment sites with very limited access of $O_2$ and protons, thereby protecting both chlorophyll and carotenoid molecules from both photo-oxidation and acid lability. The macrostructure of the region of these LHCII trimers closest to the reaction centres *in vivo* apparently acts as an effective barrier to the diffusion of oxygen, thereby decreasing the level of singlet oxygen formation where the yield of $^3$Chl would be highest (Sieffermann-Harms & Angerhofer 1998).

#### (iii) *Cytochrome* c *oxidase*

It is relevant to consider the structure of cytochrome *c* oxidase, a haem-copper-containing terminal oxidase that is located in the inner membrane of mitochondria and many bacteria. Cytochrome *c* oxidase is a redox-driven pump that couples the reduction of molecular oxygen (by four substrate protons and four electrons to two water molecules at the active site) to the translocation of four protons across the membrane that add to the electrochemical gradient across respiratory membranes for the synthesis of ATP. Since it catalyses the reverse reaction to that of PSII, cytochrome *c* oxidase may be a good comparison for PSII.

In two high-resolution X-ray structures at 2.8 Å of cytochrome oxidase, one from a soil bacterium (Iwata *et al.* 1995) and the other from bovine heart (Tsukihara *et al.* 1995), two proton pathways were identified, one for pro-

tons consumed in water formation and one for 'proton pumping'. A water channel for the directed removal of water from the catalytic site (Tsukihara *et al.* 1996) and an oxygen pathway for the entry of oxygen into the catalytic site (Riistama *et al.* 1996; Tsukihara *et al.* 1996) might also be prerequisites for functionality.

Molecular dynamic simulations of oxygen diffusion through cytochrome *c* oxidase revealed a well-defined pathway from the periplasmic space to the oxygen-binding site haem $a^3$ of the binuclear redox centre (Hofacker & Schulten 1998). The hypothesized oxygen pathway starts at the hydrophobic cavity near the membrane-exposed surface of subunit 1, close to the interface of subunit 3 where several lipid molecules are located (Hofacker & Schulten 1998), which might form an effective $O_2$ reservoir (Ferguson-Miller & Babcock 1996; Riistama *et al.* 1996). Although singlet oxygen is not generated in cytochrome oxidase, the high reduction potential of molecular oxygen may generate reduced species such as superoxide, peroxide and particularly hydroxyl radicals that are potentially damaging to the structural integrity of the mainly hydrophobic protein matrix surrounding the binuclear redox centre. The intricate coupling of dioxygen reduction to proton pumping by cytochrome *c* oxidase relies on specific pathways for the movement of oxygen to the catalytic site, the directed exit of protons and water away from the catalytic site and the pumping of protons from the matrix to the periplasmic space.

## 6. IMPLICATIONS OF THE OXYGEN ACCESSIBILITY HYPOTHESIS FOR FUNCTIONAL PSII

### (a) *Evidence for specific pathways for water, oxygen and protons within functional PSII*

Given that two proton pathways, a water channel and a tentative oxygen pathway occur in cytochrome *c* oxidase dimers, directed pathways for water entry, as well as removal of oxygen and protons from the water catalytic site, may also be required for PSII. The structural integrity of the mainly hydrophobic domains of the protein matrix surrounding the site of stable charge separation across the membrane, as well as the protein matrix surrounding the assembled manganese cluster where substrate water is bound and from which oxygen is liberated, are vital for efficient coupling of electron transfer and proton transport in PSII.

Wydrzynski *et al.* (1996) first proposed that the access of water to the oxidation catalytic site is controlled by a hydrophobic domain in the surrounding protein matrix and the production of $O_2$ is optimized by an ordered binding of the two substrate water molecules. Upon perturbation of the hydrophobic domain, for example by removal of the lumenal capping extrinsic proteins, the catalytic site becomes exposed to excess water from the bulk solvent phase and substrate water exchange is modified (Hillier *et al.* 2001). Other groups are now favouring a water pathway and stressing the importance of the hydrophobic protein structure contributing to oxygen evolution in functional PSII (e.g. Nugent *et al.* 2001; Rappaport & Lavergne 2001; Renger 2001). Interestingly, molecular dynamic simulation studies demonstrate pulsed water conduction through short hydrophobic channels of carbon nanotubes (Hummer *et al.* 2001). The dense packing of the appropriate membrane-spanning α-helices around the selective water channels of aquaporins provides a very narrow, extremely hydrophobic pore with a minimal number of solute-binding sites to facilitate rapid water transport (Sui *et al.* 2001). A largely hydrophobic water channel may be present in native PSII going through the PsbO protein to the water catalytic site within PSII, recently shown to be *ca.* 10 Å from the lumenal membrane surface without the extrinsic proteins (Zouni *et al.* 2001).

As discussed (§ 5), oxygen may be inaccessible to $P_{680}^{+}$ and if so, it must be directed out to the membrane surface from the substrate water bound at the catalytic site via a specific pathway in functional PSII. The environment around $P_{680}^{+}$ in functional PSII is almost certainly shielded from bulk water, as proton relaxation at the $P_{680}$ site is slow (Mulkidjanian 1999).

By analogy to cytochrome *c* oxidase, the need for directed pathways for the protons that are liberated from water during transitions of the S-cycle in PSII is also important. Some scientists favoured a 1 : 1 : 1 : 1 non-oscillating proton release, irrespective of pH, and suggested that proton accessibility to the bulk water phase is fast. In native PSII, however, proton generation does not occur during each transition of the S-cycle: it is oscillating and dependent on pH (e.g. Haumann & Junge 1999; Rappaport & Lavergne 2001). Clearly, the protein matrix surrounding the water catalytic site that provides proton wires or networks in functional PSII is vital. Conversely, when the proton wires or networks present in native PSII become structurally perturbed in many of the depleted PSII cores and reaction centres used for water mechanism studies, the protons are directly accessible to the bulk water phase.

### (b) *Conformational changes in thylakoid membranes in response to light*

Early studies established that marked conformational changes occur in the thylakoid membrane structure in response to light level. For example, a spectacular contraction of the height of the grana stacks of isolated thylakoids occurred under illumination compared with darkness, which was caused by thinning of thylakoid membranes (13–23%; Murakami & Packer 1970). Furthermore, there is a decrease in the spacing between appressed membranes, and a dramatic decrease (up to 30%) in the distance between opposing membranes across the lumenal space (Murakami & Packer 1970). Even at the macro level, the dynamics of PSII as a molecular machine is evident. Albertsson (1982) suggested that the enhanced attractive forces generated in the lumen in the light, coupled with the reduction of repulsive forces due to the large influx of protons into the lumen, are responsible not only for the decreased lumenal space but also for the flat structure of thylakoid membranes. The PsbO protein known as the manganese-stabilizing protein has a high content of antiparallel β-sheets and turns and a low amount of α-helices. It is therefore possible that the PsbO protein will form very different conformations under light or dark conditions. The mass of the lumenal extrinsic proteins of the oxygen-evolving complex and their relative proximity to each other at the monomer–monomer interface of PSII dimers, as well as their protrusion deep into the lumenal space, certainly provides an extremely tightly

packed, adjacent hydrophobic domain necessary for directed water, oxygen and proton movement within PSII dimers. This may partly account for the enhanced capacity for oxygen evolution by PSII dimers compared with monomers *in vivo*. The structural integrity of this extremely large hydrophobic domain may help to provide and fine-tune the postulated pathways for water, protons and oxygen, and optimize efficient oxygen evolution. In any event, both surface electrical charge at the outer and inner surfaces of thylakoid membranes, depending on light or dark conditions, must exert an effect on the structure of PSII dimers in appressed granal domains.

Horton (1999) suggests that grana formation in higher plants and green algae is a vital aspect of the regulation of light harvesting (Horton *et al.* 1996), due to a flexible arrangement of the peripheral antenna Chl *a*/*b* proteins with the core complex itself. At low light, the presence of grana may favour light harvesting and prevent a collapse of the dense array of proteins into a highly dissipative state, while in high light, the driving force of increased protonation and xanthophyll de-epoxidation may favour closer packing and enhanced NPQ (Horton 1999).

### (c) *The robustness of PSII depends partly on functional PSII* in vivo *being dimers*

The organization of the hydrophobic core, particularly at the interface of complementary monomers, provides the main driving force for specification of the native states of membrane proteins. The monomer–monomer interface of the central core of the PSII dimer provides greatly enhanced hydrophobic forces within the membrane, as well as electrostatic forces at both membrane surfaces to bind the reaction centre proteins together *in vivo*. This is strengthened, in part, by other small proteins (Zouni *et al.* 2001; Barber & Nield 2002), phosphatidylglycerol (Kruse *et al.* 2000) (not yet detected in crystals structure at the present resolution of 3.8 Å) and appropriate phosphorylation states in higher-plant PSII. Further, the proximity to each other at the complementary monomer–monomer interface in functional dimers of the lumenal extrinsic proteins that cap the water-oxidizing site, coupled to their marked protrusion into the lumenal space, may contribute to the remarkable architecture and enhanced stability of PSII dimers.

### (d) *Role of lipids in the formation of PSII dimers*

Many membrane proteins complexes exist in oligomeric states *in vivo*, although the isolated monomers are still functional. These include dimeric PSII (Zouni *et al.* 2001), cytochrome *bf* complex, cytochrome *c* oxidase and cytochrome *bc* complexes, and trimeric LHCII (Kühlbrandt *et al.* 1994) and PSI (Jordan *et al.* 2001). Most membrane protein complexes contain lipids that have structural as well as functional roles. Phosphatidylglycerol, with its unusual trans-hexadecanoic fatty acid (C16 : 1Δ3tr), has a structural and functional role in PSII, both at the reaction centre where it appears to be tightly bound to D1 protein in cyanobacteria and in the trimerization of LHCII (reviewed in Kruse *et al.* 2000). Significantly, Kruse *et al.* (2000) demonstrated that phosphatidylglycerol, but no other class of thylakoid lipids, induced dimerization of isolated PSII monomers and dimers contained more than twice the phosphatidylglycerol content of monomers. Phosphatidylglycerol binding, however, is not the only factor in these regulatory organizational changes involved in association and dissociation. *In vivo*, the interconversion between dimers and monomers and selective release and re-association of CP43 have been implicated in the D1 protein repair cycle (Baena-González & Aro 2002). Large structural changes are also controlled by reversible N-terminal phosphorylation of mainly D1, D2, PsbH and CP43 in higher plants.

### (e) *Light is needed to activate PSII: photoactivation*

One of the remarkable features of PSII is the way the redox-active manganese cluster that is required for charge accumulation is assembled by two light-driven reactions separated by a dark reaction. This photoactivation of PSII requires weak light, four manganese ions, a calcium ion, a chloride ion and a postulated role for bicarbonate (Büchel *et al.* 1999; Ananyev *et al.* 2001). Clearly, many dynamic conformational changes must occur within PSII during the initial photoactivation, when the cofactors are assembled into the oxygen-evolving complex. The tightly packed protein matrix surrounding $P_{680}$ and the oxygen pathway to direct oxygen immediately out to the membrane surface, probably need to be assembled during the initial photoactivation of PSII. The role of protein dynamics in the intricate assembly of the structure for proton-coupled electron transfer within PSII, with directed pathways for the movement of protons, water and oxygen molecules, is inextricably linked to its optimal function in the native state.

## 7. IS THE SPECIFIC OXYGEN PATHWAY DESTROYED IN STRUCTURALLY PERTURBED NON-FUNCTIONAL PSII?

Just as light is needed to activate PSII and allow assembly of the hydrophobic structural domain of the oxygen-evolving complex of native PSII (see § 6e), light is also required initially to inactivate PSII. Further light then regulates the phosphorylation of proteins in inactive PSII dimers/monomers (see § 8) that are confined to appressed granal domains under sustained high light (Anderson & Aro 1994). In effect, the photoinactivation of PSII is the converse of its photoactivation. It is well established that following photoinactivation, marked compositional changes take place progressively at both the donor and acceptor sites of non-$Q_B$-reducing PSII. Although the exact temporal sequence of these multiple dynamic compositional changes *in vivo* is not defined, they are inferred from numerous *in vitro* studies. The many studies directed towards the mechanism of water oxidation have largely concentrated on isolated PSII cores or membrane fragments following various treatments that permit removal of $Mn^{2+}$, $Ca^{2+}$ or $Cl^{-}$ ions or one or more of the lumenal extrinsic polypeptides, and in PSII mutants that lack a particular protein. Insights from this research, where the essential structural integrity of native PSII has been perturbed, show subtle and not so subtle functional changes (e.g. reviews by Nugent *et al.* 2001; Rappaport & Lavergne 2001; Renger 2001). In non-$O_2$-evolving PSII cores or reaction centres, when the Mn cluster, $Y_Z$ and $P^{+}_{680}$ are no longer protected by the PsbO, PsbP and PsbQ

extrinsic proteins and the large lumenal loops of CP47 and CP43, some $Mn^{2+}$, $Ca^{2+}$ and $Cl^-$ ions may be lost. On the acceptor side, electrons can no longer travel from $Q_A$ to $Q_B$.

Such compositional changes following the initial photoinactivation of PSII will cause profound conformational changes, resulting in a partial 'unfolding' of the D1/D2 heterodimer at both sides of the membrane and allowing direct access of water to the catalytic site, oxygen access to $P^+_{680}$ and fast access of protons to the bulk water phase. The specific water and oxygen channels, as well as proton wires or networks of functional PSII, may be destroyed.

It is intriguing to speculate that the oxidizing potential of $P^+_{680}$, formed by charge separation in non-$Q_B$-reducing PSII, may not be as high as that formed in active PSII. Whatever the factors are that contribute to the very high oxidizing potential of the four reaction centre chlorophylls (§ 2), they may be altered in inactive PSII when the structural integrity of the surrounding protein matrix has been modified and $P^+_{680}$ may then have a lower oxidizing potential. After all, water no longer has to be oxidized and, under sustained high light, it would be undesirable to permit PSII to disassemble in the granal stacked domains by oxidizing too many of the D1/D2 amino acids and thereby releasing some bound redox cofactors. If $P^+_{680}$ does indeed have a lower (less positive) oxidizing potential in photoinactivated PSIIs, the energy gap between $P_{680}/P^+_{680}$ and $Q_A/Q_A^-$ will be smaller and there may be direct transfer of the electron from $Q_A^-$ to $P^+_{680}$ in a non-radiative manner, as postulated by Krieger-Liszkay & Rutherford (1998). If so, there will be less chance of electron transfer from $Q_A^-$ back to Pheo, thereby minimizing charge recombination between $Pheo^-$ and $P^+_{680}$; this, in turn, will limit the formation of triplet $P_{680}$. We suggest that the generation of singlet oxygen in non-functional PSII *in vivo* may be lower than expected due to the likelihood of a lower oxidizing potential of $P^+_{680}$ in inactive PSII.

The profound conformational changes in photoinactivated PSII that result from the loss of redox factors are beginning to be identified in native PSII. In elegant electron microscopy and single-particle analysis, Boekema *et al.* (2000) demonstrated that removal of the PsbO protein (now known to be present as one copy per $P_{680}$; Nield *et al.* 2002). This leads to the release of some manganese, and loss of the capacity to evolve oxygen at low chloride concentration has a profound effect on the dimeric structure of PSII. An inward shift (0.9 nm) of the strongly bound LHCII complex is induced that might account for enhanced NPQ and, importantly, the monomer–monomer interactions in the central core are destabilized, leading to structural rearrangements of core monomers (Boekema *et al.* 2000). Removal of the PsbP and PsbQ extrinsic proteins, which also stabilize the manganese cluster and sequester $Cl^-$ and $Ca^{2+}$ ions close to the manganese cluster, induces a large 1.2 nm shift of the monomeric peripheral antenna protein, CP29, towards the central part of the supercomplex (Boekema *et al.* 2000).

Barber (1998) demonstrated massive oxidation by singlet oxygen of many amino acids in D1, and to a lesser extent in D2, in isolated PSII cores under very high light. Although not yet, to our knowledge, demonstrated in leaves, some oxidation by singlet oxygen of D1 presumably continues to occur in the inactive phosphorylated dimers that are confined to appressed grana regions under sustained high light (Anderson & Aro 1994). Since no PSI complexes are located in the appressed membrane domains, singlet oxygen will react locally with photoinactivated PSII, probably successively oxidizing specific amino acids of D1 and D2 without the energetically expensive disassembly of its reaction centre. If, indeed, the potential of $P^+_{680}$ that is formed by charge separation is lower in inactive PSIIs in leaves, the amount of singlet oxygen generated by inactive PSII might be limited, thereby preventing PSII disassembly within appressed membrane domains under high light in the environment. Then, the light-regulated, coordinated processes of D1 protein degradation and the simultaneous replacement with *de novo* protein synthesis occurs in non-appressed membrane domains, the region accessible to chloroplast ribosomes, but not to singlet oxygen under normal circumstances.

## 8. D1 PROTEIN REPAIR CYCLE

In the D1 protein repair cycle, non-functional dimeric PSII complexes with damaged D1 protein are phosphorylated, phosphorylated peripheral LHCII Chl *a*/*b* proteins are detached (Rintamäki *et al.* 1999) and then the phosphorylated PSII core dimer is monomerized in the appressed granal domain (Baena-González *et al.* 1999; Baena-González & Aro 2002; figure 1). The phosphorylated PSII monomers laterally migrate to non-appressed stroma thylakoids where CP43 is first dephosphorylated and then detached from the damaged PSII cores. The D1 and D2 proteins are initially dephosphorylated, damaged D1 protein is degraded and new D1 protein is simultaneously synthesized on chloroplast ribosomes, then processed and assembled with its heterodimeric partner, D2 protein. Then CP43 is re-attached and PSII monomers migrate back to appressed granal domains (Baena-González *et al.* 1999; Baena-González & Aro 2002). Thus, the highly light-regulated cycle between functional PSII dimers and non-functional PSII containing damaged D1 protein located in the appressed granal membranes, and the replacement of damaged D1 protein by D1 protein degradation and *de novo* synthesis in non-appressed membrane domains, involve marked dynamic structural and compositional heterogeneity of PSII.

## 9. CONCLUSIONS

The robustness of native higher-plant PSII depends on the complementary logic inherent in this remarkable molecular machine, which undergoes many dynamic compositional and structural changes in the complex cycle between its functional and non-functional state. Significantly, the dynamics of nearly all of these changes are modulated in response to light level. Although the detailed molecular mechanism involved in the coupling of electron and proton transfer within PSII is not yet defined, it is clear that the structural integrity of the protein matrix surrounding both the water oxidation site and the separation of charge across the membrane in functional PSII dimers is vital. The role of protein dynamics in the intricate assembly and fine-tuning of the structure for proton-coupled electron transfer within PSII, which is its photo-

activation, seems to be inextricably linked to its optimal function as a dimer in the native state. Like cytochrome *c* oxidase dimers, the directed movement of water molecules, protons and even oxygen molecules involved in the coupling of electron and proton transfer in functional PSII dimers, may be confined to specific pathways within the protein matrix. We propose that oxygen may not have direct access to $P_{680}$ in functional PSII, thereby preventing the generation of singlet oxygen from $^3P_{680}$. Further, we propose that the oxidizing potential of $P^+_{680}$ may be lower in non-functional PSII, thereby limiting the generation of singlet oxygen in inactive PSII. In effect, the inevitable photoinactivation of PSII is the converse of its photoactivation. Following the initial photoinactivation of PSII, the D1/D2 heterodimer partly unfolds, many compositional and structural changes occur in response to light and the specific pathways for directed movement of water, protons and oxygen are destroyed, prior to the D1 protein repair cycle being initiated.

Many colleagues who have contributed to the research mentioned here, particularly Dr Youn-Il Park, are acknowledged by their original references. The authors are very grateful for helpful discussion with Professor James Barber and Dr Tom Wydrzynski.

## REFERENCES

Albertsson, P.-A. 1982 Interaction between the lumenal sides of the thylakoid membrane. *FEBS Lett.* **149**, 186–190.

Ananyev, G. M., Zaltsman, L., Vasko, C. & Dismukes, G. C. 2001 The inorganic biochemistry of photosynthetic oxygen evolution/water oxidation. *Biochim. Biophys. Acta* **1503**, 52–68.

Anderson, J. M. 1999 Insights into the consequences of grana stacking of thylakoid membranes in vascular plants: a personal perspective. *Aust. J. Plant Physiol.* **26**, 625–639.

Anderson, J. M. 2001 Does functional photosystem II complex have an oxygen channel? *FEBS Lett.* **488**, 1–4.

Anderson, J. M. & Aro, E.-M. 1994 Grana stacking and protection of PSII in thylakoid membranes of higher plant leaves under sustained high irradiance: an hypothesis. *Photosynth. Res.* **41**, 315–326.

Anderson, J. M., Park, Y.-I. & Chow, W. S. 1997 Photoinactivation and photoprotection of photosystem II in nature. *Physiol. Plant* **100**, 214–223.

Anderson, J. M., Park, Y.-I. & Chow, W. S. 1998 Unifying model for the photoinactivation of photosystem II *in vivo* under steady-state photosynthesis. *Photosynth. Res.* **56**, 1–13.

Aro, E.-M., Virgin, I. & Andersson, B. 1993 Photoinhibition of photosystem II: inactivation, protein damage and turnover. *Biochim. Biophys. Acta* **1143**, 113–134.

Baena-González, E. & Aro, E.-M. 2002 Biogenesis, assembly and turnover of photoystem II units. *Phil. Trans. R. Soc. Lond.* B. **357**, 1451–1460. (DOI 10.1098/rstb.2002.1141.)

Baena-González, E., Barbato, R. & Aro, E.-M. 1999 Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II. *Planta* **208**, 196–204.

Barber, J. 1995 Molecular basis of the vulnerability of photosystem II to damage by light. *Aust. J. Plant Physiol.* **22**, 201–208.

Barber, J. 1998 Photosystem two. *Photosynth. Res.* **1365**, 269–277.

Barber, J. & Nield, J. 2002 Organization of transmembrane helices in photosystem II: comparison of plants and cyanobacteria. *Phil. Trans. R. Soc. Lond.* B **357**, 1329–1335. (DOI 10.1098/rstb.2002.1132.)

Boekema, E. J., van Breemen, J. F. L., van Roon, H. & Dekker, J. P. 2000 Conformational changes in photosystem II supercomplexes upon removal of extrinsic subunits. *Biochemistry* **39**, 12 907–12 915.

Büchel, C., Barber, J., Ananyev, G., Eshaghi, S., Watt, R. & Dismukes, C. 1999 Photoassembly of the manganese cluster and oxygen evolution from monomeric and dimeric CP47 reaction center photosystem II complexes. *Proc. Natl Acad. Sci. USA* **96**, 14 288–14 293.

Chow, W. S. 2001 The photoinactivation of photosystem II in leaves: a personal perspective. *J. Photosci.* **8**, 43–53.

Dekker, J. P. & Van Grondelle, R. 2000 Primary charge separation in photosystem II. *Biochim. Biophys. Acta* **63**, 195–208.

Diner, B. A. & Babcock, G. T. 1996 Structure, dynamics and energy conversion efficiency. In *Oxygenic photosynthesis* (ed. D. R. Ort & C. F. Yocum), pp. 213–247. Dordrecht, The Netherlands: Kluwer.

Ferguson-Miller, S. & Babcock, G. T. 1996 Heme/copper terminal oxidases. *Chem. Rev.* **96**, 2889–2907.

Haumann, M. & Junge, W. 1999 Photosynthetic water oxidation: a simplex-scheme of its partial reactions. *Biochim. Biophys. Acta* **1411**, 86–91.

Hideg, È., Kálai, T., Hideg, K. & Vass, I. 1998 Photoinhibition of photosynthesis *in vivo* results in singlet oxygen production. Detection via nitroxide-induced fluorescence quenching in broad bean leaves. *Biochemistry* **37**, 11 405–11 411.

Hillier, W., Hendry, G., Burnap, R. L. & Wydrzynski, T. 2001 Substrate water exchange in photosystem II depends on the peripheral proteins. *J. Biol. Chem.* **276**, 46 917–46 924.

Hofacker, I. & Schulten, K. 1998 Oxygen and proton pathways in cytochrome *c* oxidase. *Proteins: Struct. Funct. Genet.* **30**, 100–107.

Horton, P. 1999 Are grana necessary for regulation of light harvesting? *Aust. J. Plant Physiol.* **26**, 659–669.

Horton, P., Ruban, A. V. & Walters, R. G. 1996 Regulation of light harvesting in green plants. *A. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 655–684.

Hummer, G., Rasaiah, J. C. & Noworyta, J. P. 2001 Water conduction through the hydrophobic channel of a carbon nanotube. *Nature* **414**, 188–190.

Iwata, S., Ostermeier, C., Ludwig, B. & Michel, H. 1995 Structure at 2.8 Å resolution of cytochrome oxidase from *Paraccocus denitrificans*. *Nature* **376**, 660–669.

Jones, L. W. & Kok, B. 1966 Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. *Plant Physiol.* **41**, 1037–1043.

Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W. & Krauss, N. 2001 Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* **411**, 909–911.

Klimov, V. V., Allakhverdiev, S. I., Demeter, S. & Krasnovskii, A. A. 1979 Phaeophytin reduction in photosystem II of chloroplasts in relation to oxidation–reduction potential of the medium. *Dokl. Akad. Nauk. SSSR* **249**, 227–230.

Krieger-Liszkay, A. & Rutherford, A. W. 1998 Influence of herbicide-binding on the redox potential of the quinone acceptor in photosystem II; relevance to photodamage and phototoxicity. *Biochemistry* **33**, 17 339–17 344.

Kruse, O., Hankamer, B., Konczak, C., Gerle, C., Morris, E., Radunz, A., Schmid, G. H. & Barber, J. 2000 Phosphatidylglycerol is involved in the dimerization of photosystem II. *J. Biol. Chem.* **275**, 6509–6514.

Kühlbrandt, W., Wang, D. N. & Fujiyoshi, Y. 1994 Atomic model of plant light-harvesting complex determined by electron crystallography. *Nature* **367**, 614–621.

Mulkidjanian, A. Y. 1999 Photosystem II of green plants: on the possible role of retarded protonic relaxation in water oxidation. *Biochim. Biophys. Acta* **1410**, 1–6.

Murakami, S. & Packer, L. 1970 Protonation and chloroplast membrane structure. *J. Cell Biol.* **47**, 332–351.

Nield, J., Balsera, M., De Las Rivas, J. & Barber, J. 2002 Three-dimensional electron cryo-microscopy study of the extrinsic domains of the oxygen-evolving complex of spinach. Assignment of the PsbO protein. *J. Biol. Chem.* **277**, 15 006–15 012.

Nugent, J. H. A., Rich, A. M. & Evans, M. C. W. 2001 Photosynthetic water oxidation: towards a mechanism. *Biochim. Biophys. Acta* **1503**, 138–146.

Öquist, G., Chow, W. S. & Anderson, J. M. 1992 Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. *Planta* **186**, 450–460.

Oxborough, K. & Baker, N. R. 2000 An evaluation of the potential triggers of photoinactivation of photosystem II in the context of a Stern–Volmer model for downregulation and the reversible radical pair equilibrium model. *Phil. Trans. R. Soc. Lond.* B **355**, 1489–1498. (DOI 10.1098/rstb.2000.0709.)

Park, Y.-I., Anderson, J. M. & Chow, W. S. 1996*a* Photoinactivation of functional photosystem II and D-1 protein synthesis *in vivo* are independent of the modulation of the photosynthetic apparatus by growth irradiance. *Planta* **198**, 300–309.

Park, Y.-I., Chow, W. S., Anderson, J. M. & Hurry, V. M. 1996*b* Differential susceptibility of photosystem II to light stress depends on the capacity of photochemical and non-radiative dissipation of light. *Plant Sci.* **115**, 137–149.

Park, Y.-I., Chow, W. S., Osmond, C. B. & Anderson, J. M. 1996*c* Electron transport to oxygen mitigates against the photoinactivation of photosystem II *in vivo*. *Photosynth. Res.* **50**, 23–32.

Prásil, I., Adir, N. & Ohad, I. 1992 Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. In *Topics in photosynthesis*, vol. 11 (ed. J. Barber), pp. 295–348. Amsterdam: Elsevier.

Rappaport, F. & Lavergne, J. 2001 Coupling of electron and proton transfer in the photosynthetic water oxidase. *Biochim. Biophys. Acta* **1503**, 246–259.

Renger, G. 2001 Photosynthetic water oxidation to molecular oxygen: apparatus and mechanism. *Biochim. Biophys. Acta* **1503**, 210–228.

Rintamäki, E., Martinsuo, P. P., Pursiheimo, S. & Aro, E.-M. 1999 Cooperative regulation of light harvesting complex II photophosphorylation via the plastoquinone pool and the ferredoxin–thioredoxin system in chloroplasts. *Proc. Natl Acad. Sci. USA* **97**, 11 644–11 649.

Riistama, S., Puustinen, A., García-Horsman, A., Iwata, S., Michel, H. & Wikström, M. 1996 Channelling of dioxygen into the respiratory enzyme. *Biochim. Biophys. Acta* **1275**, 1–4.

Schatz, G. H., Brock, H. & Holzworth, A. R. 1988 Kinetic and energy model for the primary processes of photosystem II. *Biophys. J.* **54**, 397–405.

Setif, P., Hervo, G. & Mathis, P. 1981 Flash-induced absorption changes in photosystem I: radical pair or triplet state formation? *Biochim. Biophys. Acta* **638**, 257–267.

Siefermann-Harms, D. 1987 The light-harvesting and protective functions of carotenoids in photosynthetic membranes. *Physiol. Plant.* **69**, 561–568.

Siefermann-Harms, D. & Angerhofer, A. 1998 Evidence for an $O_2$-barrier in the light-harvesting chlorophyll-*a*/*b*-protein complex LHC II. *Photosynth. Res.* **55**, 83–94.

Sui, H., Han, B.-G., Lee, J. K., Walian, P. & Jap, B. K. 2001 Structural basis of water-specific transport through the AQP1 water channel. *Nature* **414**, 872–878.

Telfer, A. 2002 What is β-carotene doing in the photosystem II reaction centre? *Phil. Trans. R. Soc. Lond.* B **357**, 1431–1440. (DOI 10.1098/rstb.2002.1139.)

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R. & Yoshikawa, S. 1995 Structures of metal sites of oxidised bovine heart cytochrome *c* oxidase at 2.8 Å. *Science* **269**, 1069–1074.

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R. & Yoshikawa, S. 1996 The whole structure of the 13-subunit oxidised cytochrome *c* oxidase at 2.8 Å. *Science* **272**, 1136–1144.

Tyystjärvi, E., Kairavuo, M., Pätsikkä, E., Keränen, M., Khriachtchev, L., Tuominen, I., Guiamet, J. J. & Tyystjärvi, T. 2002 The quantum yield of photoinhibition is the same in flash light and under continuous illumination—implications for the mechanism. In *PS2001 Proc. 12th Int. Congr. of Photosynthesis*. See www.publish.csiro.au/ps2001.

Vass, I., Máté, E., Turcsányi, E., Sass, L., Szilárd, A., Nagy, F. & Sicora, C. 2002 Damage and repair of photosystem II under exposure to UV radiation. In *PS2001 Proc. 12th Int. Congr. of Photosynthesis*. See www.publish.csiro.au/ps2001.

Wydrzynski, T., Hillier, W. & Messinger, J. 1996 On the functional significance of substrate accessibility in the photosynthetic water oxidation mechanism. *Physiol. Plant* **96**, 342–350.

Zhang, L., Paakkarinen, V., van Wijk, K. J. & Aro, E.-M. 1999 Biogenesis of the chloroplast-encoded D1 protein: regulation of translation, insertion, and assembly into photosystem II. *J. Biol. Chem.* **274**, 16 062–16 067.

Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauss, N., Saenger, W. & Orth, P. 2001 Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* **409**, 739–743.

## GLOSSARY

Chl: chlorophyll
LHC: light-harvesting complex
NPQ: non-photochemical quenching
Pheo: pheophytin
PSI: photosystem I
PSII: photosystem II