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Singlet oxygen production in photosynthesis

Anja Krieger-Liszkay*

Institut für Biologie II, Biochemie der Pflanzen, Universität Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany

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Abstract

A photosynthetic organism is subjected to photo-oxidative stress when more light energy is absorbed than is used in photosynthesis. In the light, highly reactive singlet oxygen can be produced via triplet chlorophyll formation in the reaction centre of photosystem II and in the antenna system. In the antenna, triplet chlorophyll is produced directly by excited singlet chlorophyll, while in the reaction centre it is formed via charge recombination of the light-induced charge pair. Changes of the mid-point potential of the primary quinone acceptor in photosystem II modulate the pathway of charge recombination in photosystem II and influence the yield of singlet oxygen production. Singlet oxygen can be quenched by β-carotene, α-tocopherol or can react with the D1 protein of photosystem II as target. If not completely quenched, it can specifically trigger the up-regulation of the expression of genes which are involved in the molecular defence response of plants against photo-oxidative stress.

Key words: Light energy, photo-oxidative stress, photosynthesis, photosystem II, singlet oxygen.

Singlet oxygen

Living in an oxygen-rich world carries the potential risk of oxidative stress. Oxygen in the ground state is not directly a problem because it is relatively stable compared with its intermediates (peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$) and hydroxyl radicals ($OH^{\bullet}$)). The relatively stable ground state of oxygen is a triplet state with two unpaired electrons with the same spin quantum number, each located in different antibonding (π^*) orbitals. Oxygen can react by oxidizing another molecule, but, despite its high thermodynamic reactivity, its reactions are kinetically slow because of the spin restriction. Electron transfer reactions in the presence of oxygen can give rise to the production of the reactive intermediates, which themselves can produce different kinds of damage in the cell (Halliwell and Gutteridge, 1998).

In addition, the very reactive singlet oxygen can be generated by an input of energy. In this state, the spin restriction is removed and therefore the oxidizing ability of the oxygen is greatly increased. Singlet oxygen is produced by light absorption by photosensitizers and, in plants, particularly by the chlorophylls and their precursors. On the one hand chlorophylls are needed for the use of light energy in photosynthesis, on the other hand, the same molecules carry the potential danger of being a singlet oxygen producer (photosensitizer). $^1O_2$ has a short half-time of about 200 ns in cells (Gorman and Rodgers, 1992), and reacts with target molecules in the immediate neighbourhood. The possible diffusion distance of $^1O_2$ has been calculated to be up to 10 nm in a physiologically relevant situation (Sies and Menck, 1992).

In the following, the reactions leading to the production of $^1O_2$ in the antenna and reaction centres of the photosynthetic apparatus, the potential target molecules and the protection mechanism avoiding $^1O_2$ production, are described (Fig. 1). In addition, the 'useful' role of $^1O_2$ will be discussed, being not only a damaging species but also an element of signal transduction chains leading to the specific expression of stress-related genes.

Special properties of chlorophyll

Chlorophyll as the main light-absorbing pigment in the light-harvesting complex, the inner antenna, and also in the reaction centres, is very efficient in absorbing light and has the additional advantage that the excited states are long-lived enough (up to a few nanoseconds) to allow the conversion of

* Fax: +49 761 203 2601. E-mail: anja.liszkay@biologie.uni-freiburg.de

Abbreviations: chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; P_{680}, primary electron donor in PSII; Pheo, pheophytin (primary electron acceptor in PSII); PS, photosystem, Q_A, primary quinone acceptor in PSII; Q_B, secondary quinone acceptor in PSII; TEMP, 2,2,6,6-tetramethylpiperidine-1-oxyl; Tyr_Z, redox active tyrosine of the D1 protein; S_2, S_3, oxidation states of the Mn cluster.

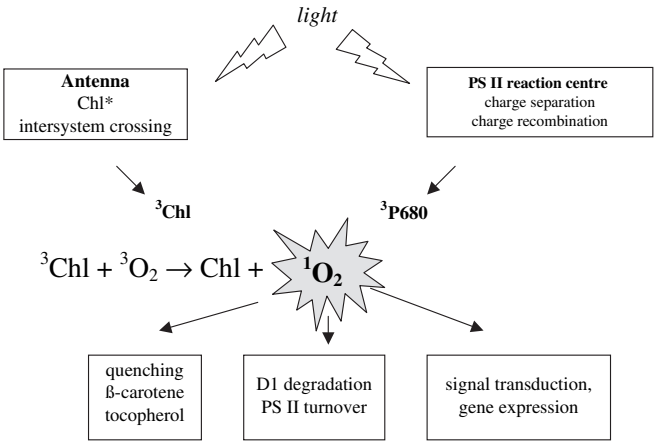

Fig. 1. Sites of production of singlet oxygen in photosynthesis and its potential targets.

the excitation energy into an electrochemical potential via charge separation. If the energy is not efficiently used, the spins of the electrons in the excited state can rephase and give rise to a lower energy excited state: the chlorophyll triplet state. The chlorophyll triplet state has an even longer lifetime (a few μs under O_2-saturated conditions) and can react with $^3O_2$ to produce the very reactive $^1O_2$ if no efficient quenchers are around. Chl triplet states may be populated in principle either directly by intersystem crossing (changing of the spin) from a singlet excited chlorophyll, or by charge recombination reactions (reversal of the charge separation and electron transfer reactions) in the reaction centres.

$^1O_2$ formation is favoured under certain physiological conditions like exposure to high light intensities or drought, leading to closure of the stomata and low CO_2 concentrations in the chloroplasts. Under such conditions the plastoquinone pool can be in a very reduced state, forward electron transport is very limited, and recombination reactions in PSII can occur. The kinetically limiting step of the photosynthetic electron transport chain is thought to be the quinol oxidation in the Q_o site of the cytochrome b_6f complex.

Quenching of chl triplet states and photoinhibition of PSII

$^1O_2$ can react with proteins, pigments, and lipids and is thought to be the most important species responsible for light-induced loss of PSII activity, the degradation of the D1 protein (protein of the reaction centre of PSII) and for pigment bleaching (for reviews on photoinhibition, see Prasil *et al*., 1992; Aro *et al*., 1993). $^1O_2$ formation *in vivo* was measured in the leaves of *Arabidopsis thaliana* by the use of a fluorescent dye (Hideg *et al*., 2001; op den Camp *et al*., 2003). Trebst and coworkers (Trebst *et al*., 2002) provided evidence that $^1O_2$ is the important damaging species during photoinhibition (i.e. the light-induced loss of PSII activity and of the D1 protein) of *Chlamydomonas reinhardtii* cells.

The dangerous triplet state of chlorophylls, which is the origin of the observed $^1O_2$, can be quenched directly by carotenoids in close proximity. The edge-to-edge distance between the two molecules must be less than the van der Waals distance (3.6 Å), i.e. the electron orbitals must have some overlap. In this spin exchange reaction, the triplet state of the carotenoid is formed which can either dissipate the excess energy directly as heat or by physical quenching via enhanced intersystem crossing with $^3O_2$ (Edge and Truscott, 1999). This possibility is given in the antenna system, but not in the reaction centre, although two β-carotene molecules are present in the PSII reaction centre (Telfer, 2002; for the location of the carotenes in the reaction centre, see Kamiya and Shen, 2003; Ferreira *et al*., 2004). In the reaction centre, the distance between the carotenes and the triplet chlorophyll is too large to allow a direct triplet quenching. The redox potential of the redox couple P_{680}/P_{680}^+ is very positive and a too close contact to the carotene would lead to the efficient oxidation of the carotene. Hence the primary function of these β-carotenes is probably the quenching of $^1O_2$ produced via the triplet state of P_{680} (Telfer, 2002). The latter was generated by charge recombination in PSII of the primary pair, P_{680}Pheo. $^1O_2$ can react with carotenoids which act as a catalyst, deactivating $^1O_2$.

Another important antioxidant located in the thylakoid membrane is α-tocopherol. Tocopherol is an efficient scavenger, which becomes oxidized when reacting with $^1O_2$ (Trebst, 2003). Trebst *et al*. (2002) showed that inhibition of tocopherol biosynthesis in *Chlamydomonas* resulted in a stimulation of light-induced loss of PSII activity and D1 protein degradation. This implies that tocopherol comes close to the site of $^1O_2$ generation in the reaction centre of PSII.

If $^1O_2$ produced via chl triplet formation in the reaction centre is not quenched by carotenoids or tocopherol, it is very probable that it reacts with the D1 protein as a target molecule. The rapid turnover of the D1 protein occurs even at low light intensities (Keren *et al*., 1995), indicating that there is always some $^1O_2$ formation even under low or moderate illumination. Singlet-oxygen-generating chemicals produce the same specific fragments of the D1 protein as are found under the conditions of acceptor side photoinhibition (Okada *et al*., 1996). The degradation of the D1 protein may be regarded as a physiological defence system to prevent uncontrolled damage of PSII. The controlled destruction of the D1 protein seems to be an attractive safety valve to detoxify $^1O_2$ directly at the place of its generation (Trebst, 2003). Damaged D1 protein is degraded and PSII is repaired efficiently by the assembly of newly synthesized D1 in the so-called D1 protein damage–repair cycle (for reviews see Prasil *et al*., 1992; Aro *et al*., 1993).

With respect to photoinhibition studies, one has to differentiate between two experimental conditions chosen

in the laboratory for *in vitro* studies: the so-called acceptor side photoinhibition which is related to the formation of $^3P_{680}$ by charge recombination in PSII, followed by $^1O_2$ production and the so-called donor side photoinhibition which occurs in PSII with a non-functional or absent water-splitting complex. In this state, photoinhibition is caused by the accumulation of highly oxidizing species like Tyr_Z^+/P_{680}^+ (Jegerschöld *et al*., 1990; Blubaugh *et al*., 1991) and is not related to $^1O_2$ production (Krieger *et al*., 1998).

Triplet chl formation by charge recombination in the reaction centre of PSII

In the reaction centre of PSII, the first detectable radical pair formed after excitation by light is $P_{680}^+Pheo^-$, with P_{680} being the primary electron donor and pheophytin the primary electron acceptor (for a recent review on PSII see Goussias *et al*., 2002; for the X-ray structure of PSII, see Zouni *et al*., 2001; Kamiya and Shen, 2003; Ferreira *et al*., 2004). In isolated PSII reaction centres which lack Q_A and a functional donor side, the primary charge pair $P_{680}^+Pheo^-$ recombines and a high yield of P_{680} triplet is formed (Durrant *et al*., 1990). In the absence of oxygen, the lifetime of the triplet state is approximately 1 ms and shortens in the presence of O_2 to approximately 30 μs. This effect, and the decrease in the stability of the reaction centre and bleaching of chl, were considered to be indirect evidence for $^1O_2$ formation (Durrant *et al*., 1990). $^1O_2$ formation was detected directly by its luminescence (Macpherson *et al*., 1993) and by EPR spin trapping (Hideg *et al*., 1994).

In isolated functional reaction centres possessing a complete and fuctional donor and acceptor side, the next step of electron transfer after the formation of the primary radical pair ($P_{680}^+Pheo^-$) leads to the reduction of the primary quinone acceptor Q_A. Subsequently, P_{680}^+ is reduced by electron donation from the redox active tyrosine Tyr_Z, which itself obtains an electron from the water oxidizing complex. Forward electron transfer is much faster than charge recombination reactions. However, charge recombination reactions can occur when the forward electron transport cannot proceed. If the primary quinone acceptor stays reduced because of a block of the forward electron transport (the so-called closed state of the reaction centre), the yield of the primary charge separation is lowered. It has been proposed that the presence of the semiquinone anion Q_A^- in closed PSII may raise the energy of the primary pair by an electrostatic interaction so that the driving force of the primary charge separation is decreased compared with open reaction centres (state of the centre with the oxidized quinone, Q_A) (van Gorkom, 1985; Schatz *et al*., 1988). In the closed reaction centre, however, if primary charge separation occurs, it is followed by recombination of the charges. Charge recombination in the primary pair will produce either the singlet ground state of P_{680} or the triplet state of P_{680}. The triplet state in the reaction centre is not localized directly on P_{680}, i.e. the chlorophyll thought to bear the positive charge, but delocalized to another monomeric chlorophyll which is tilted by 30° compared with P_{680} (van Mieghem *et al*., 1991; Kamlowski *et al*., 1996). In the presence of a large antenna (PSII *in vivo*), the yield of the primary pair formed in the presence of Q_A^- will be low (van Mieghem *et al*., 1995).

Under reducing conditions, i.e. in the presence of dithionite and light (van Mieghem *et al*., 1989) or anaerobiosis and light (Vass *et al*., 1992), Q_A becomes doubly reduced, thereby releasing the negative electrostatic effect on the energy of the primary pair, and a high yield of charge separation, recombination, and P_{680} triplet formation is observed (van Mieghem *et al*., 1989). The double reduction of Q_A and the high yield of $^3P_{680}$ formation in such centres have been suggested to have some relevance to photoinhibition (van Mieghem *et al*., 1989; Vass *et al*., 1992). The occurrence of double reduced Q_A, however, has never been shown to occur under physiologically relevant conditions.

At cryogenic temperatures (around 20 K), different from the situation at ambient temperature, the primary radical pair is formed with a high yield, irrespective of the redox state of Q_A and the yield of the triplet state $^3P_{680}$ is high both with Q_A^- and Q_AH_2 (van Mieghem *et al*., 1995). The triplet decay is much faster with Q_A^- than with Q_AH_2 present. At room temperature, the yield of the primary pair is reduced in the presence of Q_A^-, but nevertheless, a significant yield of the primary pair is found in core complexes of *Synechococcus* (about half of that found in reaction centres with double reduced Q_A) (Schlodder and Brettel, 1988; van Mieghem *et al*., 1995). Significant amounts of singlet oxygen are produced in PSII with a large antenna under continuous illumination (Hideg *et al*., 1994; Fufezan *et al*., 2002), and they are most likely linked to chl triplet formation by charge recombination of the primary pair in PSII and not to chl triplet formation in the antenna. The production of singlet oxygen via chl triplet formation by charge recombination reaction is shown by the following experiments: (i) the induction of photoinhibition by repetitive single turnover flashes and (ii) the effect of the mid-point potential of the redox couple Q_A/Q_A^- on the yield of singlet oxygen production.

Photoinhibition of PSII by repetitive flashes

If dark-adapted PSII is excited by one single turnover flash (a short saturating flash which leads to one charge separation event in the majority of reaction centres), the state $S_2Q_B^-$ is formed, with S_2 being an oxidation state of the Mn cluster, the water oxidizing complex, and Q_B, the secondary quinone acceptor. In the dark, the charges recombine via the formation of the primary radical. As already described above, charge recombination of the primary radical pair leads to the production of the singlet and the triplet state of P_{680}. The chl triplet state can react with $^3O_2$ leading to the

formation of $^1O_2$ which will damage the reaction centre. This flash-induced charge recombination reaction was exploited to investigate the mechanism of photoinhibition under low light *in vivo* (Keren *et al*., 1995) and *in vitro* (Keren *et al*., 1997). Keren and coworkers used a series of single turnover flashes, spaced with a dark interval of 32 s, and measured the degree of photoinactivation and loss of the D1 protein. The dark interval is long enough to allow charge recombination between the S_2 or S_3 state and Q_B^- to occur (the half-time of $S_2Q_B^-$ recombination is approximately 20 s; Rutherford and Inoue, 1984). When they used groups of flashes with 0.1 s spacing between the flashes in one group (for example, two flashes with 0.1 s interval and then 32 s dark interval), they observed photoinhibition after an uneven number of flashes per group and no or little photoinhibition after illumination with an even number of flashes per group. An even number of flashes produced the state $S_3Q_BH_2$ which does not recombine, while after an uneven number of flashes, charge recombination between the S_2 or S_3 state and Q_B^- occurs leading to singlet and triplet P_{680}. This shows that an overall smaller number of flashes (less light absorption in total) can be more damaging than a greater number of flashes. This study was extended using Ca^{2+}-depleted PSII preparations which were not active in water-splitting (Keren *et al*., 2000). In Ca^{2+}-depleted PSII, the Mn cluster is blocked in the S_3 state (for a review on Ca^{2+}-depleted PSII see Debus, 1992). In the single turnover flash experiments, the loss of PSII activity was measured and compared with active samples. No difference between groups of even and uneven numbers of flashes was seen in Ca^{2+}-depleted material. Using an uneven number of flashes, the activity loss was much smaller than in active samples. The yield of primary charge separation was not significantly reduced in Ca^{2+}-depleted PSII, even after several single turnover flashes (Keren *et al*., 2000), implying that a difference in the charge recombination pathway must be responsible for this phenomenon.

Influence of the redox potential of the quinone acceptor on the yield of singlet oxygen formation

In Ca^{2+}- and also in Mn-depleted PSII not only the water-splitting activity is inhibited but, in addition, the mid-point potential of the Q_A/Q_A^- redox couple is up-shifted by about 150 mV. In PSII with an active water-splitting complex, the mid-point potential of the Q_A/Q_A^- couple was found to be −80 mV (Krieger and Weis, 1992; Krieger *et al*., 1995). In centres with the high potential form of Q_A (E_m about +65 mV), forward electron flow from Q_A to Q_B is energetically disfavoured and electron transfer is therefore unlikely to occur (Fig. 2) (Johnson *et al*., 1995; Krieger *et al*., 1995; Andréasson *et al*., 1995). It was proposed that, in such centres, the shift of the mid-point potential of Q_A influences the pathway of charge recombination within the reaction centre of PSII. In active PSII, with Q_A in its normal, low potential form, charge recombination between the acceptor and the donor side proceeds with a high probability via the formation of the primary pair ($P_{680}^+Pheo^-$), resulting in the formation of singlet and triplet P_{680}. In centres with the high potential form of Q_A, the formation of the primary pair is not disfavoured (Keren *et al*., 2000) and charge recombination may occur via an alternative pathway which does not involve the formation of excited chlorophyll species (Fig. 3; see also Johnson *et al*., 1995; Rutherford and Krieger-Liszkay, 2001). As already described above, the loss of PSII activity after excitation by an uneven number of flashes was about 30% less in Ca^{2+}-depleted PSII than in active PSII. In addition, no singlet oxygen production could be measured by spin trapping EPR with TEMP under continuous illumination (Krieger *et al*., 1998). This shows that the change of the mid-point potential of Q_A is an important molecular switch for changing the charge recombination pathway within PSII. By changing the mid-point potential of Q_A from low to high potential, the formation of singlet oxygen can be avoided.

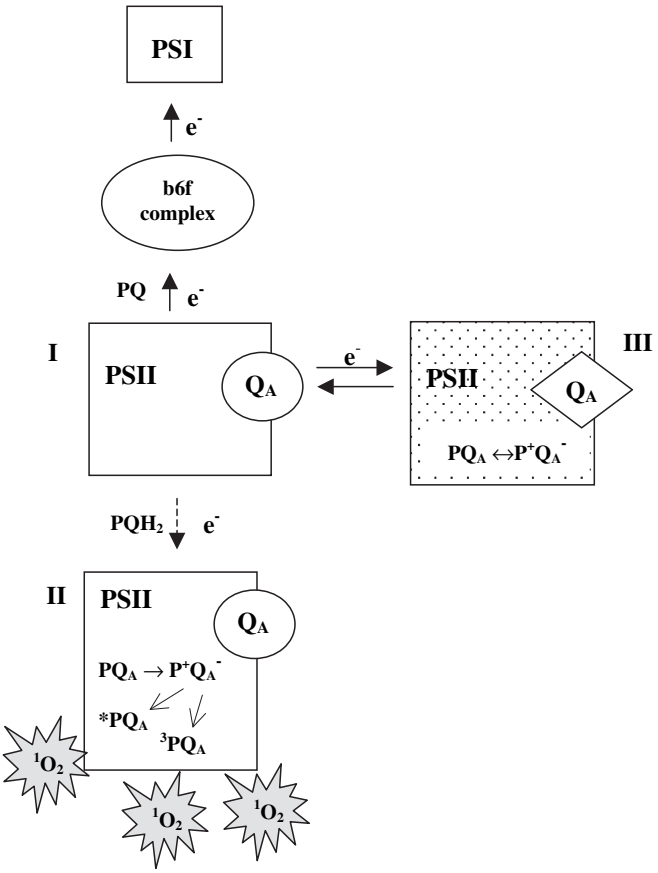

Fig. 2. Photosynthetic electron transport. Linear electron flow through PSII (I), cytochrome b_6f complex, and PSI are shown. If forward electron transport is blocked, charge recombination reactions occur in PSII leading to the formation of triplet chl which reacts with O_2 to $^1O_2$ (II). If the water-splitting complex of PSII is inactivated (prior to photoactivation or after Ca^{2+}-depletion), the mid-point potential of Q_A is shifted and charge recombination reactions are though to occur to the ground state via a safe route (III). Q_A 'low potential' is shown as a circle, Q_A 'high potential' as a diamond.

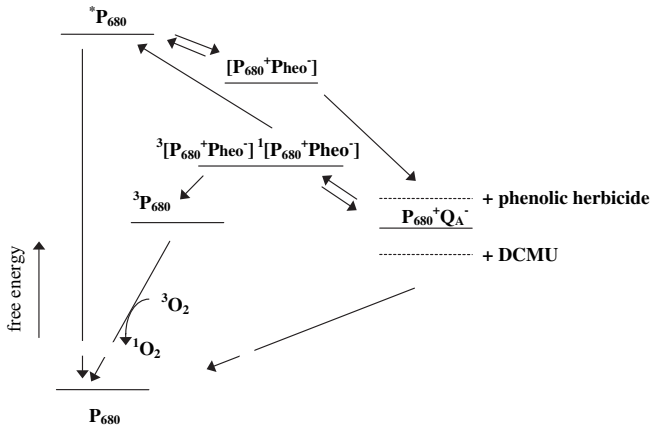

Fig. 3. Schematic diagram of the free energy levels of the states involved in recombination of the $P^+Q_A^-$ radical pair in PSII. The scheme shows the electron transfer reactions after illumination of a dark-adapted PSII. A series of radical pairs is formed, each with a slight loss of energy. The back reactions between these radical pairs require thermal activation and are thus thermodynamically disfavoured. It is assumed that the $P_{680}^+Pheo^-$ radical pair formed by back reaction from $P_{680}^+Q_A^-$ is at a lower energy level than that formed initially from $*P_{680}$ presumably through some kind of relaxation process. When the $P_{680}^+Pheo^-$ radical is formed by the back reaction from the long-lived $P^+Q_A^-$ state, there is a high probability for the formation of a triplet state $^3[P_{680}^+Pheo^-]$ because the spins had time to randomize. The triplet state of this radical pair can recombine rapidly, resulting in $^3P_{680}$. $^3P_{680}$ can react with $^3O_2$ forming $^1O_2$. The influence of herbicide binding on the mid-point potential of the redox couple Q_A/Q_A^- is shown (dashed line). When phenolic herbicides are bound, the mid-point potential of Q_A/Q_A^- is shifted by 50 mV to a more negative value and the back reaction via the $P_{680}^+Pheo^-$ radical pair is favoured. When DCMU is bound, then the mid-point potential of Q_A/Q_A^- is shifted by 50 mV to a more positive value and this back reaction is disfavoured and direct recombination to the ground state may occur.

This regulation mechanism of PSII may be of physiological importance. PSII is assembled without the Mn cluster and with Q_A in the high potential form (Johnson *et al*., 1995). In the light, during the so-called photoactivation process, the Mn cluster is assembled and the mid-point potential of Q_A is switched from high potential to the low potential form, which allows linear electron flow. In the state prior to complete assembly of the functional water-splitting complex, PSII is protected against photodamage induced by $^1O_2$ formation. Under high light conditions, the change of the mid-point potential of Q_A may also be involved in the pH-dependent control of PSII activity. In addition to the dissipation of excess energy by the formation of zeaxanthin in the antenna (for a review see Demming-Adams, 1990), the activity of the electron transfer can be altered at the level of the reaction centre of PSII (reaction centre quenching). When, in excess light, the pH in the lumen decreases below a certain threshold value, up to one Ca^{2+} per PSII can be released and the mid-point potential of Q_A is thereby switched to the high potential form (Krieger and Weis, 1992). This was demonstrated in thylakoid membranes, in which a proton gradient was maintained by ATP-hydrolysis in the dark, by measuring the chlorophyll fluorescence at the F_o-level at different redox potentials as a measure for the reduction state of Q_A (Krieger and Weis, 1993).

It is still unclear how the activity state of the water-splitting complex at the donor side is connected to the mid-point potential of the quinone at the acceptor side of PSII. One possibility is that, upon the release of Ca^{2+}, a structural change in a protein subunit of the reaction centre and especially at the Q_A binding site occurs, which could be responsible for the observed change in the mid-point potential. It might also be possible that cytochrome b_{559} mediates between the donor and acceptor sides. In inactive and non-photoactivated PSII, cytochrome b_{559} is in the low potential form and changes upon the assembly of the Mn to the high potential form, characteristic for the active PSII (for review, see Stewart and Brudvig, 1998). The change of the potential form of cytochrome b_{559} was already observed before the process of photoactivation was fully completed (Mizusawa *et al*., 1997).

Influence of herbicides on the mid-point potential of Q_A and on singlet oxygen production

The influence of different herbicides on the mid-point potentials of the primary quinone acceptor Q_A (Krieger-Liszkay and Rutherford, 1998) and single point mutations in D1 (Rappaport *et al*., 2002) or in D2 (Vavilin and Vermaas, 2000) can be used as a tool to investigate the charge recombination pathways in PSII. Binding of herbicides to the Q_B binding site of the D1 protein inhibits linear electron flow and affects the degree of photoinactivation and light-induced degradation of the D1 protein. *In vitro*, the urea herbicide DCMU and related herbicides have been reported to retard photodamage (Keren *et al*., 1995, 1997; Kirilovsky *et al*., 1994) and degradation of the D1 protein (Keren *et al*., 1995, 1997; Nakajima *et al*., 1996; Jansen *et al*., 1993; Zer and Ohad, 1995). By contrast with DCMU, phenolic herbicides, which also bind to the Q_B-binding site, have the opposite effect and stimulate the susceptibility of PSII to light (Pallett and Dodge, 1980; Nakajima *et al*., 1996) and the degradation of D1 (Jansen *et al*., 1993). Binding of these herbicides to the Q_B binding site influence the mid-point potential of Q_A. Phenolic herbicides lower the mid-point potential by approximately 45 mV and DCMU raises it by about 50 mV (Krieger-Liszkay and Rutherford, 1998). A smaller difference of 60 mV between the redox potential in the presence of bromoxynil and in the presence of DCMU was reported by Roberts *et al*. (2003) when estimated from the back reaction rate of $S_2Q_A^-$. The effect of the different types of herbicide on the mid-point potential of Q_A was not only observed for the low potential form but also for the high potential form of Q_A (Krieger-Liszkay and Rutherford, 1998). The absolute change in the mid-point potential of Q_A by these herbicides was much lower ($\pm$50 mV) than the shift induced by inactivation of the water-splitting complex (Ca^{2+}- or Mn-depletion), but it has, nevertheless, a big effect

on the yield of $^1O_2$ production. The molecular basis for the shift of the mid-point potential of Q_A by binding a herbicide to the Q_B binding pocket is not understood. FTIR spectra of Q_A obtained in the presence of a phenolic herbicide compared with DCMU indicate that the protein environment of Q_A is slightly modified by the phenolic herbicide. The change seen in the spectra is small and approximately in the range of one H-bonding (J Breton and A Krieger-Liszkay, unpublished data).

Fufezan *et al*. (2002) showed that the yield of $^1O_2$ production in the presence of a phenolic herbicide in active PSII-enriched membrane fragments (with Q_A in the low potential form) is twice as high as in the presence of DCMU. This effect is already seen at relatively low light intensities (400 μmol quanta m^{-2} s^{-1}) and the amount of $^1O_2$ produced increases linearly with increasing light intensities. In bacterial reaction centres it has been shown that the free energy gap between the $P^+Q_A^-$ radical pair and the P^+BPheo^- radical pair has a major influence on the back reaction pathway (Gunner *et al*., 1982; Gopher *et al*., 1985; Woodbury *et al*., 1986; Shopes and Wraight, 1987). When the gap is smaller than 400 meV, the back reaction via the primary pair (P^+BPheo^-) dominates, while under conditions where the gap is greater than this value, a direct recombination pathway dominates ($P^+Q_A^-\rightarrow PQ_A$). This direct recombination pathway involves electron tunnelling reactions. Based on these observations made with the bacterial reaction centre a model was proposed (Fig. 3) showing the influence of the mid-point potential of Q_A on the charge recombination pathway within PSII (Krieger-Liszkay and Rutherford, 1998; Rutherford and Krieger-Liszkay, 2001). It seems likely that the modulation of the mid-point potential of Q_A by the state of the water-splitting complex and by the herbicides will influence the free energy gap between $P_{680}^+Q_A^-$ and $P_{680}^+Pheo^-$. With DCMU it is predicted that the increase in the mid-point potential should increase the free energy gap and thereby diminish the yield of back reaction via the $P_{680}^+Pheo^-$ radical pair. By analogy to the bacterial reaction centre a direct recombination via $P_{680}^+Q_A^-$ may take place which does not result in the formation of excited singlet or triplet states of P_{680}. This model may explain the lower production of $^1O_2$ observed in the presence of DCMU in centres with low potential Q_A (Fufezan *et al*., 2002) and the absence of $^1O_2$ formation in PSII with high potential Q_A (Krieger *et al*., 1998).

On the other hand, the decreased mid-point potential of Q_A induced by phenolic herbicides should make the energy gap between $P_{680}^+Q_A^-$ and $P_{680}^+Pheo^-$ smaller and, therefore, the back reaction via the primary radical pair and the formation of P_{680} triplet more likely.

Rappaport *et al*. (2002) investigated the influence of the mid-point potential of the Pheo/$Pheo^-$ redox couple on charge recombination between $S_2Q_A^-$ and showed that the recombination rate is sensitive to the free energy gap between Pheo and Q_A. They used mutants of *Synechocystis*, in which the mid-point potential of Pheo/$Pheo^-$ was shifted by +33 mV or −74 mV compared with the wild type. The mutant with the potential shift of +33 mV showed an increase in the recombination rate ($P_{680}^+Q_A^-$) by a factor of four (measured as the decay of fluorescence after a saturating flash), while lowering of the mid-point potential slowed down the recombination by the same factor. This demonstrates that charge recombination via the formation of the primary radical pair ($P_{680}^+Pheo^-$) is a significant process in PSII reaction centres in which forward electron flow is blocked. In addition they demonstrated that the direct charge recombination pathway which does not involve the repopulation of the primary pair is significant in the mutant with the lowered mid-point potential of Pheo/$Pheo^-$. In a different set of experiments, mutations in the CD loop of the D2 protein were made in *Synechocystis* (Vavilin and Vermaas, 2000). These mutants show a temperature shift and a decrease in intensity in the thermoluminescence band originating from $S_2Q_A^-$ recombination. This was interpreted as an increase of the proportion of the direct recombination pathway of the $P_{680}^+Q_A^-$ pair which does not lead to the formation of an excited chlorophyll.

Charge recombination and chl triplet formation in the reaction centre of PSI

In PSI, under reducing conditions when the iron sulphur clusters are prereduced or when vitamin K1 is removed from the reaction centre, charge recombination reactions also occur leading to the triplet state of P_{700} at room temperature (for a review see Brettel, 1997). In PSI, the lifetime of the state $^3P_{700}$ is about 6 μs and is not shortened by $^3O_2$, indicating that P_{700} is screened from O_2 (Sétif *et al*., 1981).

$^1O_2$ production by the cytochrome b_6f complex

Illumination of the isolated cytochrome b_6f complex results in the formation of $^1O_2$, as shown by spin trapping techniques and the effects of azide, a $^1O_2$ quencher, and D_2O, which extends the lifetime of $^1O_2$ (Suh *et al*., 2000). It was shown by Suh *et al*. (2000) that the Fe-S cluster of the Rieske protein and not the cytochromes are responsible for the $^1O_2$ production in the light. The extent to which $^1O_2$ generated by the cytochrome b_6f complex contributes to photoinhibition of PSII is unclear. The cytochrome b_6f complex contains, in addition to the other cofactors, one chlorophyll with an unknown function. In principle, this chlorophyll could also be involved in $^1O_2$ formation acting as a photosensitizer.

Chlorophyll triplet and singlet oxygen production in the antenna

Chlorophyll triplet states and consecutive $^1O_2$ formation is not only produced by charge recombination in the reaction

centre, but also by intersystem crossing from a singlet excited chlorophyll in the antenna. In addition, excited states of chlorophyll precursors can lead to the formation of $^1O_2$.

In isolated protein/pigment complexes, the rate of intersystem crossing is significant (Kramer and Mathis, 1980) and the formation of triplet chlorophylls in the antenna has been shown *in vitro*. These states can be distinguished by their spectroscopic characteristics from the triplet chlorophyll in the reaction centre and they do not depend on the redox potential of the medium (Santabarbara *et al*., 2002). Two of these triplet states are probably generated in the core complex while the third one may be generated in the light-harvesting complex (Santabarbara *et al*., 2002). Formation of $^1O_2$ from isolated LHCII has been shown *in vitro* by spin trapping with TEMP (Rinalducci *et al*., 2004). Singlet oxygen may also play a role in the degradation of light-harvesting proteins. The degradation of LHCII is much slower than the degradation of the D1 protein (Lindahl *et al*., 1995), but modifications of the protein are already visible after a few hours of low intensity illumination (100 μmol quanta m^{-2} s^{-1}) in the isolated complex (Zolla and Rinalducci, 2002). Degradation of LHCII releases a large number of chl. Light-induced damage might occur from such chl which is energetically uncoupled from the antenna, and will give a high triplet chl and, therefore, possibly high $^1O_2$ yield. However, experimental evidence for the production of $^1O_2$ by triplet formation in the antenna and their involvement in the light-induced damage in PSII *in vivo* in mature leaves is still missing. In the antenna ^{3}chl will be efficiently quenched by nearby carotenoids, so that ^{3}chl, although formed with a higher probability than by charge recombination in the reaction centre, will rarely be a problem.

Production of triplet chlorophyll and $^1O_2$ may play a role during the transition from etioplasts to chloroplasts. In greening material, disorganized chlorophyll may act as a photosensitizer (Marder *et al*., 1998). Oxygen uptake by thylakoid membranes, isolated from greening material, was measured. Oxygen uptake was significantly quenched by β-carotene and α-tocopherol, indicating that production of singlet oxygen was measured by this method (Caspi *et al*., 2000). Protochlorophyllide acts also as photosensitizer, as shown for the *Arabidopsis* mutant *flu* (op den Camp *et al*., 2003). In this mutant, a protein is inactivated which plays a key role during the negative feedback control of chlorophyll biosynthesis. As a consequence, the mutant accumulates free protochlorophyllide in the dark. In this study, the production of $^1O_2$ was shown in leaves by quenching of the fluorescent dye DanePy *in vivo* after a dark–light transition of the plants (Hideg *et al*., 1998; op den Camp *et al*., 2003). It was also shown previously, by the use of herbicides which block the protoporphyrinogen oxidase, that protoporphyrin IX is a photodynamic pigment which produces high amounts of $^1O_2$ in the light (Becerril and Duke, 1989).

$^1O_2$ may also be produced by free chlorophyll and chlorophyll degradation products which may be produced during strong photoinhibition. If the light-induced damage exceeds the controlled D1 degradation and repair of PSII, further protein degradation of chl binding subunits may lead to the production of free chls, which are dangerous photosensitizers. These free chls may be bound by ELIP (Early Light Induced Proteins) proteins (Adamska, 1997) or by proteins like WSCP (Water Soluble Chlorophyll Protein) (Schmidt *et al*., 2003). The binding of chl to the WSCP reduces the yield of $^1O_2$ production by a yet unknown mechanism. This protein is an unusual chl-binding protein in the sense that it does not bind carotenoids, but, nevertheless, efficiently protects bound chl against photodegradation and reduces the yield of $^1O_2$ production.

Gene expression in response to $^1O_2$ formation

There are recent reports in the literature that, as a response to $^1O_2$ production, genes are specifically up-regulated which are involved in the molecular defence response of the plant against photo-oxidative stress (Leisinger *et al*., 2001; op den Camp *et al*., 2003; B Fischer, personal communication). Leisinger *et al*. (2001) showed that, in the presence of photosensitizers like Rose Bengal, a glutathione peroxidase homologous gene from *Chlamydomonas* is transcriptionally up-regulated by $^1O_2$, while the mRNA level of this gluthathione peroxidase is only weakly expressed by exposure to superoxide or peroxide. Op den Camp *et al*. (2003) used the flu mutant of *Arabidopsis* to show that the accumulation of protochlorophyllide and thus the high yield of $^1O_2$ formation by transferring these plants from dark to light rapidly activated a number (70) of genes. By contrast, other reactive oxygen species like superoxide did not rapidly up-regulate the expression of these genes. In the *flu* mutant, $^1O_2$ is produced peripherally at the membrane surface and can, therefore, react with compounds of the stroma. Under natural conditions, $^1O_2$ will be produced within the reaction centre of PSII and will react with different target molecules than in this mutant. Fischer, however, using inhibitors of the photosynthetic electron transport, studied $^1O_2$ formation in PSII and only found a significant up-regulation of the glutathione peroxidase homologous gene from *Chlamydomonas* (B Fischer, personal communication).

The question arises how an extremely short-lived molecule like $^1O_2$ can give rise to a signal that can be transmitted to the nucleus to regulate gene expression. Some other reactive oxygen species like superoxide or peroxide have been shown to act directly as second messengers in the regulation of expression of the oxidative stress response genes such as gutathione peroxidases, glutathione-*S*-transferases, and ascorbate peroxidase (for reviews see Mullineaux *et al*., 2000; Vranová *et al*., 2002). Because of the short lifetime of $^1O_2$, it can be excluded that

it oxidizes a component of a signal transduction chain directly. Instead reaction products originating either from the D1 protein degradation or products of chlorophyll degradation can be envisaged as signal molecules. It has been shown that chlorophyll precursors like Mg-protoporphyrin IX can act as a signalling molecule in a signalling pathway between the chloroplasts and the nucleus (Strand *et al*., 2003). By analogy, one can also speculate that a chlorophyll degradation product such as pheophytin, chlorophyllide, or pheophorbide (for chl degradation, see Matile *et al*., 1999) may act as a signalling molecule. Such a molecule could be transported out of the chloroplast to the cytosol by an ABC protein where it mediates a signal to the nucleus to regulate the expression of genes. It has been shown that a functional ABC protein is required for the transport of protophorhyrin IX (Møller *et al*., 2001). It was also shown that an ABC transporter in the tonoplast membrane can transport chlorophyll catabolites to the vacuole (Lu *et al*., 1998), making it likely that such a transport mechanism is also present in the chloroplast envelope membrane.

Alternatively, lipid peroxides may function as signalling molecules because unsaturated fatty acids are the preferred targets of $^1O_2$. However, no increase in $^1O_2$-mediated non-enzymatic lipid peroxidation could be found in the *flu* mutant, which accumulates protochlorophyllide and shows a higher yield of $^1O_2$ formation than wild-type plants (Op den Camp *et al*., 2003). Linolenic acid was rapidely oxidized upon illumination of the *flu* mutant, but the oxidation patterns observed were indicative for enzymatic oxidation and not for non-enzymatic oxidation by $^1O_2$. In general, fatty acid-derived signals may be involved in signalling pathways connected with cell death and the expression of stress-related genes (Weber, 2002).

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References

Adamska I. 1997. ELIPs—light induced stress proteins. *Physiologia Plantarum* **100,** 794–805.

Andréasson LE, Vass I, Styring S. 1995. Ca^{2+}-depletion modifies the electron transfer on both donor and acceptor sides in photosystem II from spinach. *Biochimica et Biophysica Acta* **1230,** 155–164.

Aro EM, Virgin I, Andersson B. 1993. Photoinhibition of photosystem II. inactivation, protein damage and turnover. *Biochimica et Biophysica Acta* **1143,** 113–134.

Becerril, JM, Duke, SO. 1989. Protoporphyrin IX content correlates with activity of photobleaching herbicides. *Plant Physiology* **90,** 1175–1181.

Blubaugh DJ, Atamian M, Babcock GT, Golbeck JH, Cheniae GM. 1991. Photoinhibition of hydroxylamine-extracted photosystem II membranes: identification of the sites of photodamage. *Biochemistry* **30,** 7586–7597.

Brettel K. 1997. Electron transfer and arrangement of the redox factors in photosystem I. *Biochimica et Biophysica Acta* **1318,** 322–373.

Caspi V, Malkin S, Marder JB. 2000. Oxygen uptake photosensitized by disorganized chlorophyll in model systems and thylakoids of greening barley. *Photochemistry and Photobiology* **7,** 441–446.

Debus RJ. 1992. The manganese and calcium ions of photosynthetic oxygen evolution. *Biochimica et Biophysica Acta* **1102,** 269–352.

Demmig-Adams B. 1990. Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta* **1020,** 1–24.

Durrant JR, Giorgi LB, Barber J, Klug DR, Porter G. 1990. Characterization of triplet-states in isolated photosystem II reaction centres—oxygen quenching as a mechanism for photodamage. *Biochimica et Biophysica Acta* **1017,** 176–175.

Edge R, Truscott TG. 1999. Carotenoid radicals and the interaction of carotenoids with active oxygen species. In: Frank HA, Young AJ, Britton G, Cogdell RJ, eds. *Advances in photosynthesis: the photochemistry of carotenoids*, Vol. 8. Dordrecht: Kluwer, 223–234.

Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S. 2004. Architecture of the photosynthetic oxygen-evolving center. *Science* **303,** 1831–1838.

Fufezan C, Rutherford AW, Krieger-Liszkay A. 2002. Singlet oxygen production in herbicide-treated photosystem II. *FEBS Letters* **532,** 407–410.

Gopher Y, Blatt A, Schonfeld M, Okamura MY, Feher G, Montal M. 1985. The effect of an applied electric field on the charge recombination kinetics in reaction centers reconstituted in planar bilayers. *Biophysical Journal* **48,** 311–320.

Gorman AA, Rodgers MA. 1992. Current perspectives of singlet oxygen detection in biological environments. *Journal of Photochemistry and Photobiology* **14,** 159–176.

Goussias C, Boussac A, Rutherford AW. 2002. Photosystem II and photosynthetic oxidation of water: an overview. *Philosophical Transactions of the Royal Society of London B* **357,** 1369–1381.

Gunner MR, Tiede DM, Prince RC, Dutton PL. 1982. Quinones as prosthetic groups in membrane electron transfer proteins. Systematic replacement of the primary ubiquinone of photochemical reaction centers with other quinones. In: Trumpower BL, ed. *Functions of quinones in energy conserving systems*. New York: Academic Press, 265–269.

Halliwell B, Gutteridge JM. 1998. *Free radicals in biology and medicine*, 3rd edn. Oxford: Oxford University Press.

Hideg E, Spetea C, Vass I. 1994. Singlet oxygen production in thylakoid membranes during photoinhibition as detected by EPR spectroscopy. *Photosynthesis Research* **39,** 191–199.

Hideg E, Kalai 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,** 11405–11411.

Hideg EE, Ogawa K, Kalai T, Hideg K. 2001. Singlet oxygen imaging in *Arabidopsis thaliana* leaves under photoinhibition by excess photosynthetically active radiation. *Physiologia Plantarum* **112,** 10–14.

Jansen MA, Depka B, Trebst A, Edelman M. 1993. Engagement of specific sites in the plastoquinone niche regulates degradation of the D1 protein in photosystem II. *Journal of Biological Chemistry* **268,** 21246–21252.

Jegerschöld C, Virgin I, Styring S. 1990. Light-dependent degradation of the D1 protein in photosystem II is acceleerated after inhibition of the water-splitting reaction. *Biochemistry* **29,** 6179–6186.

Johnson GN, Rutherford AW, Krieger A. 1995. A change in the mid-point potential of the quinone Q_A in photosystem II associated

with photoactivation of oxygen evolution. *Biochimica et Biophysica Acta* **1229,** 201–207.

Kamiya N, Shen JR. 2003. Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7 Å resolution. *Proceedings of the National Academy of Sciences, USA* **100,** 98–103.

Kamlowski A, Frankemöller I, van der Est A, Stehlik D, Holzwarth AR. 1996. Evidence for the delocalization of the triplet state $^3P_{680}$ in the $D_1D_2cytb_{559}$-complex of photosystem II. *Berichte der Bunsen Gesellschaft* **100,** 2045–2051.

Keren N, Gong H, Ohad I. 1995. Oscillations of reaction centre II-D1 protein degradation *in vivo* induced by repetitive flashes. *Journal of Biological Chemistry* **270,** 806–814.

Keren N, Berg A, van Kan PJM, Levanon H, Ohad I. 1997. Mechanism of photosystem II inactivation and D1 protein degradation at low light intensities: the role of electron back flow. *Proceedings of the National Academy of Sciences, USA* **94,** 1579–1584.

Keren N, Ohad I, Rutherford AW, Drepper F, Krieger-Liszkay A. 2000. Inhibition of photosystem II activity by saturating single turnover flashes in calcium-depleted and active photosystem II. *Photosynthesis Research* **63,** 209–216.

Kirilovsky D, Rutherford AW, Etienne AL. 1994. Influence of DCMU and ferricyanide on photodamage in photosystem II. *Biochemistry* **33,** 3087–3095.

Kramer H, Mathis P. 1980. Quantum yield and rate of formation of the carotenoid triplet state in photosynthetic structures. *Biochimica et Biophysica Acta* **593,** 319–329.

Krieger A, Weis E. 1992. Energy-dependent quenching of chlorophyll-*a*-fluorescence: the involvement of proton–calcium exchange at photosystem 2. *Photosynthetica* **27,** 89–98.

Krieger A, Weis E. 1993. The role of calcium in the pH-dependent control of photosystem II. *Photosynthesis Research* **37,** 117–130.

Krieger A, Rutherford AW, Johnson GN. 1995. On the determination of the redox mid-point potential of the primary quinone acceptor, Q_A, in photosystem II. *Biochimica et Biophysica Acta* **1229,** 193–201.

Krieger A, Rutherford AW, Vass I, Hideg E. 1998. Relationship between activity, D1 loss, and Mn binding in photoinhibition of photosystem II. *Biochemistry* **37,** 16262–16269.

Krieger-Liszkay A, Rutherford AW. 1998. Influence of herbicide binding on the redox potential of the quinone acceptor in photosystem II: relevance to photodamage and phytotoxicity. *Biochemistry* **37,** 17339–17344.

Leisinger U, Rüfenacht K, Fischer B, Pearo M, Spengler A, Zehnder AJB, Eggen RIL. 2001. The glutathione peroxidase homologous gene from *Chlamydomonas reinhardtii* is transcriptionally up-regulated by singlet oxygen. *Plant Molecular Biology* **46,** 395–408.

Lindahl M, Yang DH, Andersson B. 1995. Regulatory proteolysis of the major light-harvesting chlorophyll *a/b* protein of photosystem II by light-induced membrane associated enzymic system. *European Journal of Biochemistry* **213,** 503–509.

Lu YP, Li ZS, Drozdowicz YM, Hortensteiner S, Martinoia E, Rea PA. 1998. AtMRP2, an *Arabidopsis* ATP binding cassette transporter able to transport glutathione *S*-conjugates and chlorophyll catabolites: functional comparisons with Atmrp1. *The Plant Cell* **10,** 267–82.

Macpherson AN, Telfer A, Truscott TG, Barber J. 1993. Direct detection of singlet oxygen from isolated photosystem II reaction centres. *Biochimica et Biophysica Acta* **1143,** 301–309.

Marder JB, Droppa M, Caspi V, Raskin VI, Horváth G. 1998. Light-dependent thermoluminescence from thylakoids of greening barley leaves: evidence for involvement of oxygen radicals and free chlorophyll. *Physiologia Plantarum* **104,** 713–719.

Matile P, Hortensteiner S, Thomas H. 1999. Chlorophyll degradation. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 67–95.

Mizusawa N, Miyao M, Yamashita T. 1997. Restoration of the high-potential form of cytochrome b_{559} of photosystem II occurs via a two-step mechanism under illumination in the presence of manganese ions. *Biochimica et Biophysica Acta* **1318,** 145–158.

Møller SG, Kunkel T, Chua NH. 2001. A plastidic ABC protein involved in intercompartmental communication of light signaling. *Genes and Development* **15,** 90–103.

Mullineaux P, Ball L, Escobar C, Karpinska B, Creissen G, Karpinski S. 2000. Are diverse signalling pathways integrated in the regulation of *Arabidopsis* antioxidant defense gene expression in response to excess excitation energy? *Philosophical Transactions of the Royal Society of London B* **355,** 1531–1540.

Nakajima Y, Yoshida S, Ono T. 1996. Differential effects of urea/triazine-type and phenol-type photosystem II inhibitors on inactivation of the electron transport and degradation of the D1 protein during photoinhibition. *Plant Cell Physiology* **37,** 673–680.

Op den Camp RGL, Przybyla D, Ochsenbein C, *et al.* 2003. Rapid induction of distinct stress responses after the release of singlet oxygen in *Arabidopsis*. *The Plant Cell* **15,** 2320–2332.

Okada K, Ikeuchi M, Yamamoto N, Ono T, Miyao M. 1996. Selective and specific cleavage of the D1 and D2 proteins of photosystem II by exposure to singlet oxygen: factors responsible for the susceptibility to cleavage of proteins. *Biochimica et Biophysica Acta* **1274,** 73–79.

Pallet KE, Dodge AD. 1980. Studies into the action of some photosynthetic inhibitor herbicides. *Journal of Experimental Botany* **31,** 1051–1066.

Prasil O, Adir N, Ohad I. 1992. Dynamics of photosystem II: mechanism of photoinhibition and recovery process. In: Barber J, ed. *Topics in photosynthesis, the photosystems: structure, function and molecular biology*, Vol. 11. Amsterdam: Elsevier, 220–250.

Rappaport F, Guergova-Kuras M, Nixon PJ, Diner BA, Lavergne J. 2002. Kinetics and pathways of charge recombination in photosystem II. *Biochemistry* **41,** 8518–8527.

Roberts AG, Gregor W, Britt, RD, Kramer DM. 2003. Acceptor and donor-side interactions of phenolic herbicides in photosystem II. *Biochimica et Biophysica Acta* **1604,** 23–32.

Rinalducci S, Pedersen JZ, Zolla L. 2004. Formation of radicals from singlet oxygen produced during photoinhibition of isolated light-harvesting proteins of photosystem II. *Biochimica et Biophysica Acta* **1608,** 63–73.

Rutherford AW, Inoue Y. 1984. Oscillation of delayed luminescence from PSII: recombination of $S_2Q_B^-$ and $S_3Q_B^-$. *FEBS Letters* **165,** 163–170.

Rutherford AW, Krieger-Liszkay A. 2001. Herbicide-induced oxidative stress in photosystem II. *Trends in Biochemical Science* **26,** 648–653.

Santabarbara S, Bordignon E, Jennings RC, Carbonera D. 2002. Chlorophyll triplet states associated with photosystem II in thylakoids. *Biochemistry* **41,** 8184–8194.

Schatz GH, Brock H, Holzwarth AR. 1988. Kinetic and energetic model for the primary processes in photosystem II. *Biophysics Journal* **54,** 397–405.

Schlodder E, Brettel K. 1988. Primary charge separation in closed Photosystem II with a lifetime of 11 ns: flash-absorption spectroscopy with O_2-evolving Photosystem II complexes from *Synechococcus*. *Biochimica et Biophysica Acta* **933**, 22–34.

Schmidt K, Fufezan C, Krieger-Liszkay A, Satoh H, Paulsen H. 2003. Recombinant water-soluble chlorophyll protein from *Brassica oleracea* var. *botrys* binds various chlorophyll derivatives. *Biochemistry* **42,** 7427–7433.

Sétif P, Hervo G, Mathis P. 1980. Flash-induced absorption changes in photosystem I, radical pair or triplet state formation? *Biochimica et Biophysica Acta* **638,** 257–267.

Shopes RJ, Wraight CA. 1987. Charge recombination from the P^+QA^- state in reaction centres from *Rhodopseudomonas viridis*. *Biochimica et Biophysica Acta* **893,** 409–425.

Sies H, Menck CF. 1992. Singlet oxygen induced DNA damage. *Mutation Research* **275,** 367–375.

Stewart DH, Brudvig GW. 1998. Cytochrome b_{559} of photosystem II. *Biochimica et Biophysica Acta* **1367,** 63–87.

Strand A, Asami T, Alonso J, Ecker JR, Chory J. 2003. Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrin IX. *Nature* **421,** 79–83.

Suh HJ, Kim CS, Jung J. 2000. Cytochrome b_6f complex as an indigenous photodynamic generator of singlet oxygen in thylakoid membranes. *Photochemistry and Photobiology* **71,** 10–109.

Telfer A. 2002. What is β-carotene doing in the photosystem II reaction centre? *Philosophical Transactions of the Royal Society of London B* **357,** 1431–1440.

Trebst A. 2002. A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardii*. *FEBS Letters* **516,** 156–160.

Trebst A, Depka B, Hollander-Czytko H. 2003. Function of β-carotene and tocopherol in photosystem II. *Zeitschrift für Naturforschung* **58c,** 609–620.

van Gorkom HJ. 1985. Electron transfer in photosystem II. *Photosynthesis Research* **6,** 97–112.

van Mieghem F, Brettel K, Hillmann B, Kamlowski A, Rutherford AW, Schlodder E. 1995. Charge recombination reactions in photosystem II. 1. Yields, recombination pathways, and kinetics of the primary pair. *Biochemistry* **34,** 4798–4813.

van Mieghem FJE, Nitschke W, Mathis P, Rutherford AW. 1989. The influence of the quinone-iron electron acceptor complex on the reaction centre photochemistry of photosystem II. *Biochimica et Biophysica Acta* **977,** 207–214.

van Mieghem FJE, Satoh K, Rutherford AW. 1991. A chlorophyll tilted 30° relative to the membrane in the photosystem II reaction center. *Biochimica et Biophysica Acta* **1058,** 379–385.

Vass I, Styring S, Hundal T, Koivuniemi A, Aro EM, Andersson B. 1992. Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced QA species promote chlorophyll triplet formation. *Proceedings of the National Academy of Sciences, USA* **89,** 1408–1412.

Vavilin DV, Vermaas WFJ. 2000. Mutations in the CD-loop region of the D2 protein in *Synechocystis* sp. PCC 6803 modify charge recombination reaction pathways in photosystem II *in vivo*. *Biochemistry* **39,** 14831–14838.

Vranová E, Inzé D, van Breusegem F. 2002. Signal transduction during oxidative stress. *Journal of Experimental Botany* **53,** 1227–1236.

Woodbury NW, Parson WW, Gunner MR, Prince RC, Dutton PL. 1986. Radical pair energetics and decay machanisms in reaction centers containing anthraquinones, naphthoquinones or benzoquinones in place of ubiquinone. *Biochimica et Biophysica Acta* **851,** 16–22.

Weber H. 2002. Fatty acid-derived signals in plants. *Trends in Plant Sciences* **7,** 217–224.

Zer H, Ohad I. 1995. Photoinactivation of photosystem II induces changes in the photochemical reaction center II abolishing the regulatory role of the QB site in the D1 protein degradation. *European Journal of Biochemistry* **231,** 448–453.

Zolla L, Rinalducci S. 2002. Involvement of active oxygen species in degradation of light-harvesting proteins under light stresses. *Biochemistry* **41,** 14391–14402.

Zouni A, Witt HT, 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.