Review

Function of β-Carotene and Tocopherol in Photosystem II

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New and known structural and functional insights in the role of β-carotene and of α-tocopherol in photosytem II are reviewed. A concept is presented connecting the failure of P680 triplet quenching by β-carotene with the formation of singlet oxygen and its scavenging in the turnover of the D1 protein and by tocopherol in the maintenance of PS II structure and function.

Key words: Chlorophyll Triplet, D1 Protein Turnover, Singlet Oxygen

Introduction

There is a multitude of carotenoids in plants. But only very few are of significance in the basic function in photosynthesis of eukaryotes: β-carotene (sometimes partially α-carotene) and the xanthophylls lutein, violaxanthin (with its high light-dependent deepoxidation to zeaxanthin) and neoxanthin (Cogdell and Frank, 1996; Yamamoto and Bassi, 1996; Govindjee, 1999). Only two carotenoids are essential: β-carotene and violaxanthin. Mutational deletions show that lutein can be replaced by violaxanthin (Pogson *et al.*, 1996). Neoxanthin can be deleted altogether (Croce *et al.*, 1999). β-Carotene is a component of the photosystem reaction centers and core antenna, xanthophylls are restricted to the outer antenna and light harvesting proteins. The function of carotenoids is primarily as accessory pigments (Cogdell and Frank, 1996; Yamamoto and Bassi, 1996). As effective quenchers of chlorophyll triplets and of singlet oxygen they are furthermore part of protective systems for overexcitation. Zeaxanthin, derived from violaxanthin in high light is also a singlet chlorophyll quencher (Cogdell and Frank, 1996; Yamamoto and Bassi, 1996; Demmig-Adams, 1990). All functional carotenoids in the photosynthetic system in the thylakoid membrane are protein bound (Green, 1996). In that they are also structural components in the stability of transmembrane proteins, in several cases obligatory for assembly of protein complexes (see below).

This review concentrates on the two β-carotenes in the reaction center of photosystem II and considers the evidence for a divergent role of these two carotenes which has its origin in their orientation in the reaction center proteins D1 and D2. A just defined role of tocopherol in PS II protection is introduced. Data in the literature on reaction center chlorophyll triplets, their quenching by oxygen but not by carotene, singlet oxygen in D1 protein turnover and in scavenging by tocopherol are combined in a concept for the sequence of events in the protection of PS II in high light.

The location of β-carotene on the reaction center polypeptides as cause for ineffective quenching of chlorophyll triplets and formation of singlet oxygen

The reaction center of PS II – comprised of the two homologous polypeptides D1 and D2 – contains binding sites for six chlorophylls a, two pheophytins, two plastoquinones, one iron and four manganese atoms and one cytochrome b_{559} (Satoh, 1996). The two proteins furthermore bind two β-carotenes. The principal protein folding and the binding of the pigments on the D1 and D2 protein can be modeled like the reaction center with the homologous peptides L and M and equivalent pigments of purple bacteria (Trebst, 1986). There a high resolution atomic structure is long known (Deisenhofer *et al.*, 1984) and is recently refined for the orientation of the carotenoid (Lancaster and Michel, 1999). The present atomic structure of crystallized photosystem II (Rhee *et al.*, 1998; Zouni *et al.*, 2001) specifies its homology to the

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purple bacteria. It allows to place the chlorophylls, pheophytins, Fe and Mn and the cytochromes. However, its resolution of 3.8 Å do not indicate the orientation of the two β-carotenes in PS II reaction center. The prediction from functional properties is that the carotenes are between 5 and 14 Å distance away from chlorophylls. This is based on two principal experimental observations. A carotene can be oxidized by the reaction center via a monomeric chlorophyll, *i.e.* a distance required of about 14 Å (for reviews see below). But they are not closer than 4 Å, as the carotenes do not quench the triplet of P680 (see details below) which requires orbital overlap and a close distance of chlorophyll and carotene. While carotenoids do effectively quench chlorophyll triplets and singlet oxygen in the antenna chlorophyll binding proteins and in the light harvesting system (Cogdell and Frank, 1996) they do not quench P680 triplets in the reaction center (detailed below). If the ineffectiveness of β-carotene to do so in the PS II reaction center is a matter of distance (as discussed by van Gorkom and Schelvis, 1993; Telfer and Barber, 1995), it could be resolved when their binding niche can be defined.

It was an early experimental observation by Mathis (Mathis *et al.*, 1979; Satoh and Mathis, 1981; Takahashi *et al.*, 1987) that no carotene triplet could be detected in the chlorophyll triplet quench in the reaction center of PS II. This is again an observation made by Barber (Durrant *et al.*, 1990; Telfer *et al.*, 1994) more recently. This differs dramatically from bacteriochlorophyll triplet quenching by a carotenoid in the reaction center of purple bacteria (Schenk *et al.*, 1984). In purple bacteria the triplet states are on the dimeric reaction center bacteriochlorophylls P870 and then migrate to the monomeric bacteriochlorophyll B_B on the M-side. At this M-side bacteriochlorophyll B_B the one carotenoid in purple bacteria RC is located and it is close for orbital overlap explaining the observed occurrence of a carotenoid triplet (Schenk *et al.*, 1984). Whereas the orientation of the one carotenoid in the purple bacterial RC is well defined from the X-ray structure (Lancaster and Michel, 1999), no stringent assignment was possible as to the location of the two β-carotenes on the D1 and D2 reaction center proteins (Zouni *et al.*, 2001). The orientation of carotenes in a PS II structure can be modeled alike the purple bacteria carotenoid with – as there are two – a symmetric orientation of one carotene each close to either monomeric chlorophyll on the D1 and D2 protein (Xiong *et al.*, 1998). Indeed extraction data indicated this symmetrical attachment of β-carotene, one each bound to the D1 and D2 protein (Tomo *et al.*, 1997). However, spectroscopic data were interpreted as excitonic coupling with a close proximity and even a 90 degree overlap of the two carotenes to each other, *i.e.* both carotenes are bound in a shared binding niche (Newell *et al.*, 1991; Telfer *et al.*, 1991; Telfer, 2002; Telfer *et al.*, 2003). A β-carotene oxidation by PS II (see Tracewell *et al.*, 2001; Faller *et al.*, 2001; Diner and Rappoport, 2002 for reviews and details) involves cytochrome b_{559} and is possible when the donor side had been inactivated. The carotene oxidation may occur via a monomeric chlorophyll, and it is rereduced by cytochrome b_{559} (Buser *et al.*, 1992; Tracewell *et al.*, 2001). But this chlorophyll oxidation may be on a side branch in equilibrium with carotene (see Faller *et al.*, 2001; Diner and Rappoport, 2002). The present X-ray data show this cytochrome b_{559} located on the D2 protein side of the PS II complex (Zouni *et al.*, 2001). It follows that at least one β-carotene is on the D2 protein, possibly in van der Waals distance to chlorophyll as in the M protein in the purple bacteria. But then where is the second β-carotene? Is it at the D1 protein or also at the D2 protein?

The triplet states of reaction center chlorophylls do not arise from singlet excited states by intersystem crossing as in the antenna chlorophylls (Cogdell and Frank, 1996) but in recombination reactions in the first steps of charge separation to pheophytin and Q_A (Rutherford and Krieger-Liszkay, 2001; Diner and Rappoport, 2002). This mechanism allows influence of the redox state of an electron carrier – in case of PS II it is plastoquinone – on the triplet yield. For more details see Diner and Rappoport (2002). This is discussed in particular by Fufezan *et al.* (2002) for the influence of herbicide binding on the triplet P680 (and singlet oxygen) yield. The triplet yield can be very high with 30 % of the radical pair (Telfer, 2002).

The low temperature triplet state of P680 resides on a monomeric chlorophyll (van Mieghem *et al.*, 1991; Noguchi *et al.*, 2001) with a 30 degree angle to the membrane plane (van Mieghem *et al.*, 1991). At higher temperatures the triplet state is

delocalized to another monomeric chlorophyll, perpendicular to the membrane (Kamlowski *et al.*, 1996) possibly P_A or P_{D1} = the chlorophyll on the D1 protein side of a possible chlorophyll dimer. Assuming, of course, that P680 and its triplets are on the active arm of electron flow then both these states are on chlorophylls on the D1 protein. The failure to quench these triplets by carotene would indicate that both the first and the second β-carotene as phrased and discussed in the last paragraph above are not on the D1 protein. The early observations by polarized EPR (van Mieghem *et al.*, 1991) on the P680 triplet state on a monomeric chlorophyll are part of the ongoing discussion on the identity of P680 (see Diner and Babcock, 1996; Dekker and van Grondelle, 2000; Barber and Archer, 2001; Diner and Rappoport, 2002 for detailed reviews). The uncertainty is whether the primary charge separation is on a monomeric rather than on a dimeric chlorophyll as in the purple bacteria and whether there is no dimeric chlorophyll at all. And is this monomeric chlorophyll one of the accessory chlorophylls B or one of the perpendicular ones. Indeed the two perpendicular chlorophylls in PS II reaction center appear further apart (Rhee *et al.*, 1998; Zouni *et al.*, 2001; see also Kamiya and Shen, 2003) than the dimeric state in the purple bacteria (Deisenhofer *et al.*, 1984). It would result in a situation in which the two P680 triplet states in PS II as well as P680 itself are shared by the monomeric chlorophyll [which indeed has a 30 degree angle in the X-ray structure (Zouni *et al.*, 2001)] in the active arm *i. e.* chlorophyll Chl_{D1} (or B_A) and the monomeric chlorophyll P_{D1} (see Fig. 1) somewhat detached from the second perpendicular P_{D2} so that no dimeric chlorophyll is formed. This sharing of excitation between chlorophylls in a reaction center in a multimer model (Dekker and van Grondelle, 2000) might be similar to that seen in the purple bacteria reaction center in a superexchange process between several bacteriochlorophylls in the primary charge separation (see Diner and Babcock, 1996). With these results and proposals the clarification for the failure of carotenes in chlorophyll triplet quenching in PS II comes again to the question of distance of the carotenes from the chlorophylls that are candidates for P680 (Telfer and Barber, 1995).

Very recent interpretations of new X-ray data of a crystallized PS II preparation from the cyanobacterium *Thermosynechococcus vulcanus* (Kamiya and Shen, 2003) appear to solve the uncertainty as to the carotene binding sites. Remarkably even at the present relative low resolution the authors were indeed able to trace the two carotenes. Perhaps with some uncertainty as to details the β-carotenes are assigned to be both on the D2 protein one in a trans and one in a *cis* configuration (Kamiya and Shen, 2003). Being close to a monomeric chlorophyll Chl_{D2} (or B_B) and to cytochrome b_{559} it allows – in distance – the oxidation reactions in the cyt b_{559} cycle as discussed above. But significant for the intention of this review is that in this assignment of (Kamiya and Shen, 2003) the carotenes are too far away for quenching of triplet states of the D1 protein chlorophylls. This is shown in Fig. 1 based on the data of Kamiya and Shen (2003).

It should be noted also that in PS I there is no carotenoid close to the reaction center P700 chlorophylls according to the high resolution X-ray structure of PS I (Jordan *et al.*, 2001). This seems surprising, but then there is very little triplet production in PS I recombination reactions. The 96 chlorophylls attached to the antenna part on the two subunits for P700 binding do have the proper close distance to the 22 carotenoids for triplet quenching (Jordan *et al.*, 2001).

This state of the structural orientation of carotene in the PS II reaction center indicates a reduced functional significance of the two β-carotenes (Telfer, 2002). Nevertheless they are essential for the assembly and maintenance of PS II. PS II will not assemble *de novo* (Karapetyan *et al.*, 1991; Markgraf and Oelmüller, 1991) if no β-carotene is available. This is against PS I and the purple bacteria reaction center that do assemble in the absence of carotenoids. Also in the turnover of the D1 protein newly synthesized β-carotene is obligatory for the repair arm of the cycle (Trebst and Depka, 1997). This structural role for carotenes in PS II (Havaux, 1998) is part of the starting awareness (Zhang *et al.*, 2003) that single lipophilic components are of significance in hydrophobic protein helical interactions and in the tilt against each other.

Accepting the perhaps still somewhat putative assignment of carotene orientation in the PS II reaction center by Kamiya and Shen (2003) the un-

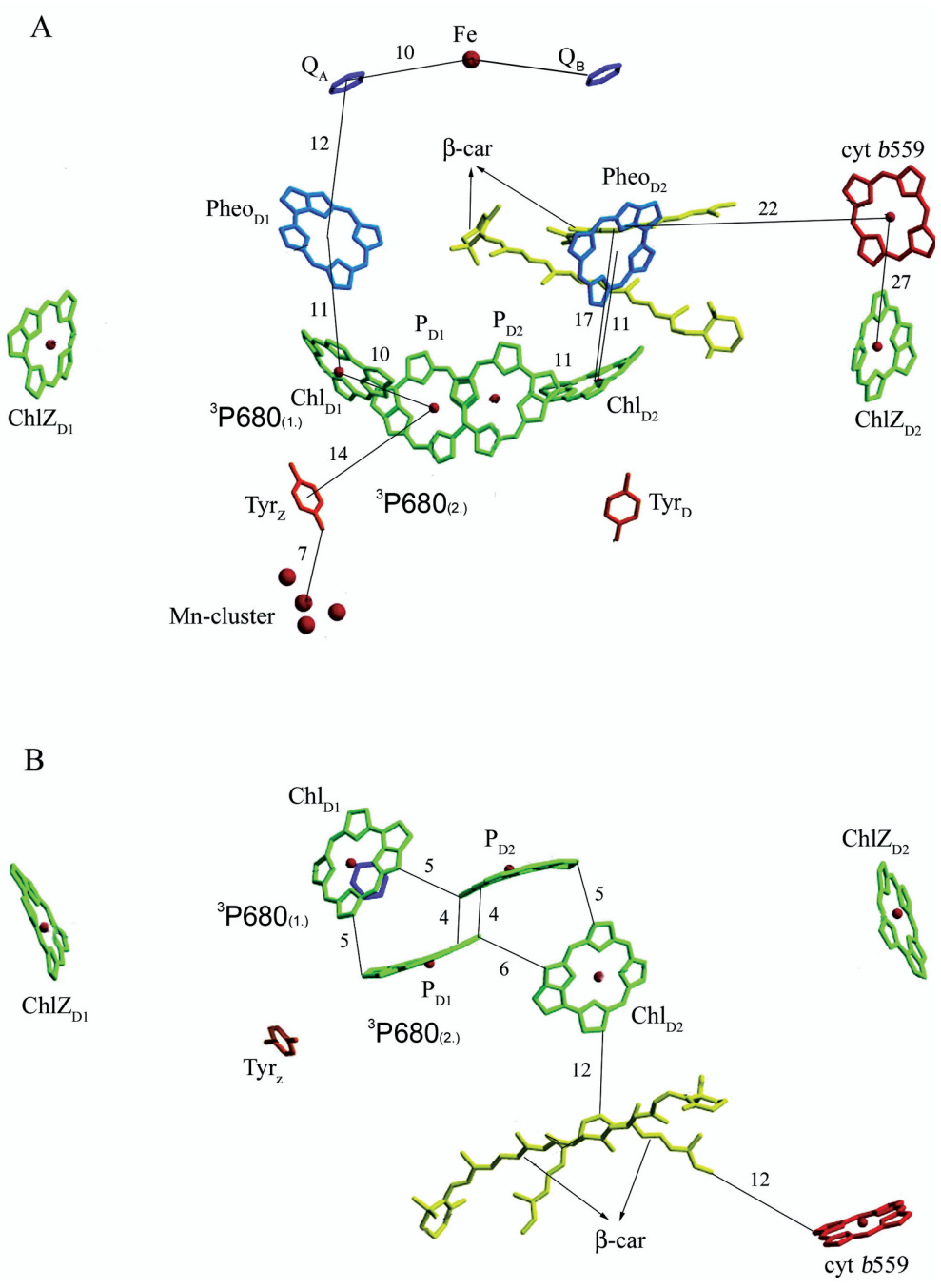

Fig. 1. Location of P680 triplets among the reaction center pigments of PS II. The low temperature triplet state $^3P680_{(1.)}$ on Chl_{D1} (van Mieghem *et al.*, 1991) and the delocalized state at higher temperatures called here $^3P680_{(2.)}$ on P_{D1} of the D1 protein (Kamlowski *et al.*, 1996) are marked on the recent resolution of crystallographic data by Kamiya and Shen (2003). Numbers on arrows are distances given by Kamiya and Shen (2003). The two carotenes (β-car) in the reaction center were assigned by Kamiya and Shen (2003) to the D2 protein. Q_B was added to the figure although not visible in the X-ray structure of Kamiya and Shen (2003).
The figure is adapted from Fig. 3 of Kamiya and Shen (2003). A is the view in the membrane plane, B from the lumenal side perpendicular to the membrane plane.

certainty about the binding site of the second β-carotene in PS II appears no longer of that interest and the consequences had been anyway of concern to only a few. Now it is just a modification in carotenoid binding: two carotenes in PS II instead of one in purple bacteria. They are located on equivalent polypeptides: the D2 or M protein side respectively of the reaction center complex. Also the shift of ^{3}P680 towards the D1 protein side appears a subtle change. But the consequence of those small differences in carotene and RC orientation seem harsh, as they make the P680 triplet (see Durrant *et al.*, 1990; Rutherford and Krieger-Liszkay, 2001; Furfezan *et al.*, 2002 and discussion above) a producer of reactive oxygen and photosynthesis stress responsive. β-Carotene may partly quench that singlet oxygen, as shown (Telfer *et al.*, 1991; Telfer *et al.*, 1994; Telfer, 2002) as the carotenoid protect in the bacterial system (Cogdell *et al.*, 2000).

It has been pointed out (van Gorkom and Schelvis, 1993; see Telfer and Barber, 1995; Telfer, 2002) that there is reason not to place carotene in quenching distance to P680 on the D1 protein. The very high oxidation potential of P680 would easily oxidize a close carotene, which in turn would disturb the reduction of $P680^+$ by the watersplitting system. Consequently alternatives for the quenching of ^{3}P680 had to be invented. If oxygen were allowed to quench then the intermittent $^1O_2$ had to be removed efficiently at the point of its generation before it can diffuse away and oxidize uncontrolled. Even the distance of the point of singlet oxygen generation to carotene quenching of $^1O_2$ may be too far and diffusion too slow and undirected and therefore incomplete. Two reaction sequences for an immediate scavenging of $^1O_2$ at ^{3}P680 can be described: the turnover of the D1 protein and the presence of tocopherol.

The turnover of the D1 protein of PS II

Singlet oxygen formation from P680 triplet is well documented (Macpherson *et al.*, 1993; Telfer *et al.*, 1994; Hideg *et al.*, 1994; Hideg *et al.*, 1998; Rutherford and Krieger-Liszkay, 2001; Fufezan *et al.*, 2002). It seems accepted [see reviews on singlet oxygen in photoinhibition (Barber and Andersson, 1992; Aro *et al.*, 1993; Mishra *et al.*, 1994; Vener *et al.*, 1998; Trebst, 1999; Ohad *et al.*, 2000) – see also an extended review on the many results and concepts in photoinhibition by I. Ohad in the forthcoming millennium edition of Photosynthesis Research] that singlet oxygen generated by PS II triplet quenching induces the degradation of one of the reaction center polypeptides, the D1 protein (Keren *et al.*, 1997). In steady state photosynthesis conditions the D1 protein is continuously degraded but also continuously resynthesized, processed and reassembled so that the photosynthesis rate remains unimpaired (Mattoo *et al.*, 1989; Keren *et al.*, 1995 and reviews above). No other subunits of PS II are affected, *i.e.* the effect of $^1O_2$ is on the point of its formation on the D1 protein. This constant "rapid D1 protein turnover", long known (Ohad and Arntzen, 1984; Mattoo *et al.*, 1989), occurs even at low light intensities (Keren *et al.*, 1995; Jansen *et al.*, 1999). From the results it follows that there is P680 triplet and singlet oxygen formation at any light intensity. Note, that there is no consistent change in PS II activity and amount during D1 turnover although it includes a reaction with $^1O_2$. The redoxstate of Q_A determines triplet and $^1O_2$ formation and effect on the D1 protein (Vass *et al.*, 1992; Melis, 1999; Rutherford and Krieger-Liszkay, 2001; Fufezan *et al.*, 2002). Which amino acid side group might be involved in which way is not known yet. An oxidation of the D1 protein is observed in mass spectrometry by additional oxygen atoms introduced into the protein (Sharma *et al.*, 1997; Barber and Sharma, 2000). The primary cleavage of the protein may be close to this site (Barbato *et al.*, 1991). An external protease cuts at the DE loop (Greenberg *et al.*, 1987), conveyed there by a conformational change. This conformational change exposing the cleavage site (Trebst, 1991) may not require singlet oxygen, when the PS II donor site is disturbed otherwise (Krieger *et al.*, 1998). At increasing light intensity and enhanced D1 protein degradation, the rate of translation and assembly of new D1 protein may no longer compensate the degradation rate; then with the D1 protein also the other chlorophyll binding subunits of PS II disappears (see reviews cited above). As a consequence of protein degradation the chlorophylls bound to the PS II subunits are set free. They may be caught by ELIP proteins [indeed induced in high light (Adamska, 1997)] or are degraded by the ubiquitous chlorophyll degradation pathway

(Hörtensteiner *et al.*, 1998; Matile *et al.*, 1999), where mutational deletions indicate their obligatory role. If not removed the free chlorophyll or chlorophyllide therefrom are starting their disastrous photodynamic action with a cascade of oxygen radicals, leading to membrane disintegration and pigment bleaching. Often masked in experimental studies on "damage" in the D1 protein turnover this secondary ROS (= reactive oxygen species) formation and reactions are mistaken as an effect of the primary $^1O_2$. When studying primary reactions in D1 protein turnover and photoinhibition it is essential to stop further exposure to light when bleaching sets in. Only then can the primary protective modes be studied.

These mechanisms in keeping PS II activity constant place a different and new emphasis on the physiological significance of the rapid turnover of the D1 protein. The turnover should not be considered a damage/repair cycle that should be avoided. D1 Protein degradation is not a cause of damage and of concern but rather a desired part of a physiological defense system to prevent uncontrolled and not repairable damage of PS II. Its existence is obligatory, not for removing "damaged" D1 protein, but for scavenging singlet oxygen, particularly efficient as it reacts at the site of generation of $^1O_2$ at the P680 chlorophyll binding site in the reaction center on the D1 protein.

The role of tocopherol

The role of α-tocopherol (= vitamin E) as an antioxidant is well known for plants (Fryer, 1992; Munné-Bosch and Alegre, 2002) and humans. But a more specific function could not be formulated so far. Tocopherol(s) are located in the thylakoid membrane and plastoglobuli (Lichtenthaler, 1968; Tevini and Lichtenthaler, 1970) suggesting a protective role for photosynthesis. Its concentration in the plant is light dependent. Tocopherol is an effective singlet oxygen scavenger, being oxidized to tocopherylquinone, irreversibly as the chromanol ring is opened (Neely *et al.*, 1988). The reaction proceeds via the 8-hydroperoxy-α-tocopherone, which is hydrolyzed to the quinone (see Fig. 2). (A note here on the chemistry of oxidations by singlet oxygen. The primary reaction is a two electron oxidation and one oxygen or a peroxy group is introduced into the target. Oxygen to water is four electrons. Therefore H_2O_2 may be formed in PS II in high light? See below).

Also the 2,3-epoxy-tocopherylquinone is formed (Neely *et al.*, 1988).

Recently both mutants in and inhibitors of the biosynthetic pathway to tocopherol yielded for the first time insights in the protection mode of tocopherol as specific antioxidant in plants. We made use of bleaching herbicides, shown by Schulz *et al.* (1993) to interfere with the hydroxyphenylpyruvate dioxygenase in the homogentisic acid pathway to plastoquinone and tocopherols (see Fig. 3). By a controlled inhibition of the biosynthesis of tocopherol in *Chlamydomonas reinhardtii* we obtained a PS II inactivation caused by tocopherol deficiency in the algae in high light (Trebst *et al.*, 2002). By this interrupting of the resynthesis it is demonstrated that there is a light dependent turnover of tocopherol in photosynthesis. This tocopherol turnover in photosynthesis remained unnoticed as there was no way to disrupt the system. Tocopherol is oxidized by PS II in the light via singlet oxygen, but at the same time it is resynthesized. Blocking the synthesis makes the turnover visible and measurable (Trebst *et al.*, 2002). Inhibition of tocopherol synthesis lowers its concentration in the thylakoid membrane (Trebst *et al.*, 2002). When degraded in high light and not replaced it can no longer scavenge singlet oxygen produced in the P680 triplet quenching. The D1 protein degradation is then much increased and PS II activity

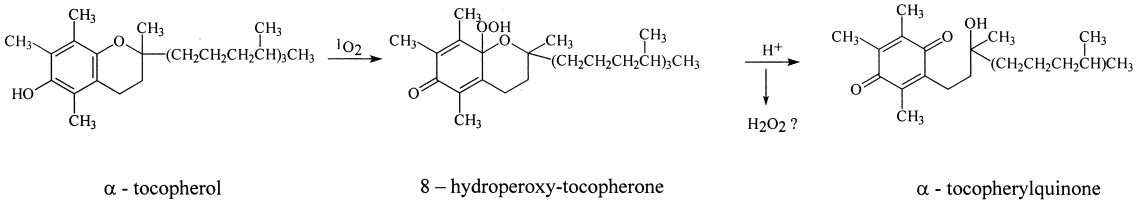

Fig. 2. Oxidation of tocopherol to tocopherylquinone by singlet oxygen.

is lowered and eventually fully inactive. The tocopherol turnover is relatively minor and oxidation is low in weak light and hence is fully compensated for by resynthesis. However, its oxidation is high in strong light, likely further increased by additional stress and may then no longer be compensated by resynthesis. Following the fate of the D1 protein in these experiments with the inhibitors of the homogentisic pathway in the green alga *Chlamydomonas reinhardtii* (Trebst *et al.*, 2002) we observed that as the tocopherol pool decreases, the D1 protein is degraded and eventually after 2 h strong light has disappeared. Then, of course, the PS II activity is zero. Longer illumination leads to bleaching of the chlorophyll. This bleaching effect is indeed the phytotoxic response in higher plants where the inhibitors of the dioxygenase are in use as commercial herbicides, like sulcotrione, pyrazolynate and isoxaflutole (Pallett, 2000). [This herbicidal bleaching effect had at first been attributed to inhibition of plastoquinone rather than tocopherol biosynthesis. Because plastoquinone is most likely the immediate oxidant in the phytoene-desaturase and therefore plastoquinone deficiency might limit carotene biosynthesis with the consequence of bleaching (Norris *et al.*, 1995; Pallett, 2000). Though, of course, aware of a tocopherol deficiency (Pallett *et al.*, 1998), a role of tocopherol in photosynthesis and in bleaching could not be anticipated at that time.] The same chlorophyll bleaching has been observed very early in a tocopherol deficient mutant of *Scenedesmus obliquus* (Bishop and Wong, 1974) and recently in a tobacco mutant with an antisense gene in the geranylgeraniol-phytylation sequence of tocopherol biosynthesis (Graßes *et al.*, 2001). At high light the tocopherol content gets low and the plants bleach out in this mutant. Other mutants, however, in the cyclase or methylation steps in cyanobacteria and in *Arabidopsis* seem to indicate little significance for tocopherol (Norris *et al.*, 1995; Schledz *et al.*, 2001; Collakova and DellaPenna, 2001; Porfirova *et al.*, 2002; Dähnhardt *et al.*, 2002).

The quantification of singlet oxygen formation in steady state PS II function and a ratio of its quenching by carotene and possibly protein bound histidines and by its scavenging by the D1 protein and by tocopherol have not yet been worked out. It appears that there is a ratio of D1 protein turnover to that of tocopherol turnover already at low light intensities (Trebst *et al.*, 2002). At a low stress situation D1 protein turnover may be sufficient to keep singlet oxygen low. Then the system may not need tocopherol, although consumed when available. This is possibly the reason why in some tocopherol deficient mutants photosynthesis remains intact. The tocopherol function is not necessarily to prevent D1 protein degradation altogether. Rather its role is to supplement in stress situations. The phenotype of tocopherol deficiency in the mutants above may therefore not be easily predicted or there might be even none.

Putting together the phenomena discussed so far: triplet formation of P680 in the recombination from Q_A^-, failure of the carotenes to quench them because of distance and no orbital overlap, of singlet oxygen formation, of D1 protein degradation and of tocopherol scavenging one arrives at a sequence of events like in the scheme in Fig. 3.

Downregulation of photosystem II activity

On diminished demand for reducing power from the electron transport system by the sink systems of the plant, the acceptor sites of the photosystems get (over-)reduced. It has been proposed, that the redoxstate of plastoquinone is sensor and signal for responses in the expression system (Pfannschmidt *et al.*, 2002) and in enzymic activities (Vener *et al.*, 1998; Allen, 2002). In light acclimation (Gilmore and Govindjee, 1999), for example, the ratio and the amount of the photosystems respond to the changes in incoming light intensity. The control of the LHCP kinase by the redoxstate (of Q_o) in the cytochrome b/f complex is a prime example for the adjustment of an enzyme activity (Gal *et al.*, 1997; Zito *et al.*, 1999) reducing in this case the antenna size. Another is photoinhibition (Vener *et al.*, 1998), the system discussed here. As the Q_A, Q_B and the PQ pool change their redoxstate (and likely further properties, like standard potential, see above) the singlet/triplet ratio of the primary radical pair (P680+/Pheo-) lowers and more P680 triplet is formed. As discussed above singlet oxygen is formed. At low concentrations of $^1O_2$ the D1 protein turnover – induced by $^1O_2$ – takes care of this singlet oxygen. It can do so as long as the D1 protein resynthesis and reassembly rate of PS II is sufficiently high to compensate for the loss of protein. Increasing $^1O_2$

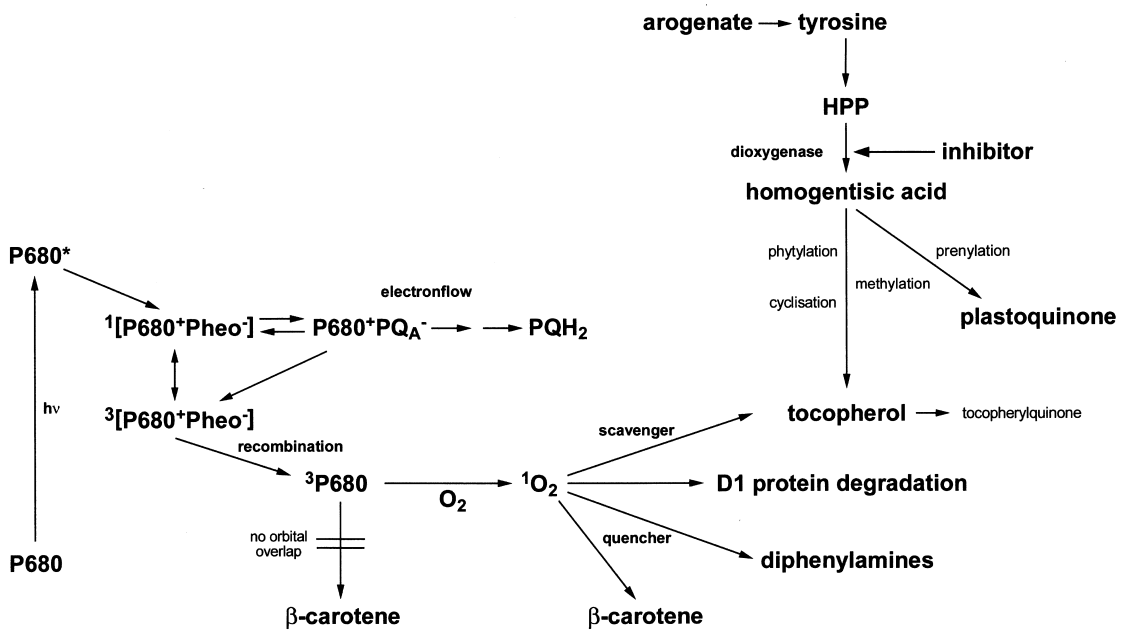

Fig. 3. Sequence of events in the high light response of PS II. Overreduction of the electron flow system at plastoquinone leads to increased triplet formation in the recombination reaction. As the location and orientation of the triplets states and the β-carotenes in the PS II reaction center do not allow orbital overlap, oxygen may react with the triplet and form singlet oxygen. Singlet oxygen may diffuse to the carotenes and be quenched. But at the site of generation it is scavenged by tocopherol being oxidized to tocopherylquinone, but it also induces D1 protein degradation. Limiting tocopherol biosynthesis by an inhibitor at the dioxygenase level leads to depletion of the tocopherol pool and increased D1 protein degradation. A chemical quencher like diphenylamine prevents all singlet oxygen induced oxidations and PS II inactivation (Trebst *et al.*, 2002).

concentrations (*i. e.* increasing light and ^{3}P680) are taken care of by tocopherol scavenging, as discussed above. Again this is a turnover situation. As long as the resynthesis rate of tocopherol (as well as its diffusion rate to the donor side of PS II) can compensate the loss of tocopherol in the $^1O_2$ scavenging (oxidation of tocopherol to tocopherylquinone), PS II remains active. If not compensated the D1 protein and then also the other subunits are degraded as PS II is not reassembled. The amount of PS II complexes decreases. This is desired when the ratio of PS II to PS I should be lowered.

The downregulation of PS II is thus controlled by:

1. the redox state and redox potential of PQH_2 leading to P680 triplets and singlet oxygen;

2. by the rate of protein synthesis of the D1 protein subunit;

3. by the reassembly rate of PS II, limited not only by the availability of the protein subunits, but also of cofactors, metals and pigments, to be reattached to form a new reaction center; lack of carotene for reassembly is one example of disappearance of PS II during D1 protein turnover (Trebst and Depka, 1997);

4. by the rate of tocopherol turnover and synthesis in the homogentisic acid pathway.

The many steps involved may allow fine tuning.

In redox regulations ROS are discussed as messengers in signal pathways (Ryter and Tyrell, 1998; Baier and Dietz, 1999; Foyer and Noctor, 2000; Rodermel, 2001; Dietz *et al.*, 2002). It may be considered whether the singlet oxygen derived from P680 triplet is also in signal transduction – as $^1O_2$ is a consequence of the redoxstate of plastoquinone as sensor – in spite of its short lifetime and the effective scavenging reactions introduced here.

Besides overreduction of PS II high light intensities affect also PS I. Here the system responds by forming superoxide radical anion (reviewed by Asada, 1999). There are several mechanisms for

taking care of $O_2^{\cdot -}$: superoxide dismutase, ascorbate and glutathione reductases and peroxidases, which are reviewed well (Asada, 1999; Foyer and Noctor, 2000). There might be also damage to PS I at the primary acceptors (Sonoike, 1996; Ohad *et al.*, 2000), but this seems not of physiological relevance and specific repair mechanisms for affected iron centers and turnover of a protein subunit are not known.

Also senescence and induction of apoptosis are regulated processes and not the result of "damage" [see Chrost *et al.* (1999) for role of tocopherol]. The sequences of reactions sketched here are likely to play a major role in mechanism and signaling.

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