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Mini Review

Quality Control of Photosystem II

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Photosystem II is particularly vulnerable to excess light. When illuminated with strong visible light, the reaction center D1 protein is damaged by reactive oxygen molecules or by endogenous cationic radicals generated by photochemical reactions, which is followed by proteolytic degradation of the damaged D1 protein. Homologs of prokaryotic proteases, such as ClpP, FtsH and DegP, have been identified in chloroplasts, and participation of the thylakoid-bound FtsH in the secondary degradation steps of the photodamaged D1 protein has been suggested. We found that cross-linking of the D1 protein with the D2 protein, the α-subunit of cytochrome b_{559}, and the antenna chlorophyll-binding protein CP43, occurs in parallel with the degradation of the D1 protein during the illumination of intact chloroplasts, thylakoids and photosystem II-enriched membranes. The cross-linked products are then digested by a stromal protease(s). These results indicate that the degradation of the photodamaged D1 protein proceeds through membrane-bound proteases and stromal proteases. Moreover, a 33-kDa subunit of oxygen-evolving complex (OEC), bound to the lumen side of photosystem II, regulates the formation of the cross-linked products of the D1 protein in donor-side photoinhibition of photosystem II. Thus, various proteases and protein components in different compartments in chloroplasts are implicated in the efficient turnover of the D1 protein, thus contributing to the control of the quality of photosystem II under light stress conditions.

Key words: D1 protein — OEC subunits — Photosystem II — Protease — Protein degradation — Reactive oxygen species.

Abbreviations: CP43, antenna-chlorophyll-binding protein of photosystem II having a relative molecular mass of 43 kDa; D1 and D2, reaction center-binding proteins of photosystem II; FT-IR, Fourier transform infrared; OEC33, -24 and -18, extrinsic proteins of the oxygen-evolving complex having relative molecular masses of 33, 24 and 18 kDa, respectively; P680, primary electron donor of photosystem II; Q_A and Q_B, primary and secondary plastoquinone electron acceptors of photosystem II, respectively; Tyr_Z and Tyr_D, redox-active tyrosines of the D1 and D2 proteins, respectively.

Introduction

Photosystem II is the site of water oxidation in photosynthesis. Light energy, captured by antenna chlorophylls and carotenoids, is finally transferred to the photosystem II reaction center, where photo-induced charge separation takes place between the primary electron donor and acceptor. The photosystem II complex is one of the multi-subunit complexes in thylakoids, and is composed of more than 30 proteins encoded by both nuclear and chloroplast genomes (Barber 1998). Of these protein subunits, the chloroplast-encoded intrinsic proteins D1 and D2, both having a molecular mass of 32 kDa, are of particular importance. This is because the redox components required for the photochemical reaction, namely the primary electron donor P680, the primary electron acceptor pheophytin, the secondary electron donor Tyr Z and the secondary quinone electron acceptors Q_A and Q_B, are ligated to or located in these protein subunits (Fig. 1). The D1 and D2 proteins each consist of five membrane-spanning helices and interconnecting loops, with the N-terminal and C-terminal tails being exposed to the stroma and thylakoid lumen, respectively. The cluster of four Mn atoms accumulates the oxidative equivalents produced by photosystem II photochemical reactions and is responsible for water oxidation. The Mn cluster appears to be ligated to the D1 protein at the lumenal side. Three nucleus-encoded extrinsic proteins of photosystem II having apparent molecular masses of 33, 24 and 18 kDa, referred to as OEC33, 24 and 18, respectively, bind to the core of photosystem II at the lumenal surface to regulate the water oxidation process.

One of the pronounced features of photosystem II is its susceptibility to damage by excessive light. Plants are exposed to various levels of light stress under natural conditions. Plants use complex and unique strategies to avoid becoming light-stressed and thus avoid damage. However, once light stress overwhelms these strategies, then chloroplasts have to repair damage caused by excessive light. Under excessive illumination by visible light, the D1 protein of photosystem II becomes a target for photodamage, which inhibits electron transport in photosystem II. Damaged D1 protein is degraded rapidly (Ohad et al. 1984), and is replaced by a newly synthesized protein (Mattoo et al. 1984, Ohad et al. 1984). This process is most important in overcoming the photoinhibition of photosystem II. Degradation of the photodamaged D1 protein by proteases was suggested by earlier work (Aro et al. 1990, Virgin et al. 1990). Light-induced degradation of the D1 protein was reported with isolated photosystem II core complexes (Virgin et al. 1990,

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Virgin et al. 1991). It was later reported that a serine-type protease inhibitor diisopropylfluorophosphate inhibits the degradation of the D1 protein and at the same time binds covalently to CP43 of the photosystem II complex (Salter et al. 1992). Based on these observations, it was proposed that CP43 possesses a serine-type protease activity, which suggests the possibility of participation in the degradation of the D1 protein. However, light-induced degradation of the D1 protein was also observed in a photosystem II preparation lacking CP43, and it was claimed that autodegradation of the D1 protein takes place within the photosystem II complexes (Shipton and Barber 1991). By contrast, a non-enzymatic mechanism involving the chemical cleavage of the D1 protein by reactive oxygen was reported by Miyao and coworkers (Miyao et al. 1995, Okada et al. 1996). Although these previous studies, collectively, have elucidated the process of cleavage, degradation and replacement of the D1 protein (Andersson and Aro 1997), the proteolytic process itself is unclear. The degradation of the D1 protein has been observed primarily with photosystem II-enriched samples, and degradation pathways of the D1 protein involving a membrane bound protease(s) have been demonstrated as described above. In this review, I summarize the recent progress of investigations on the degradation of the D1 protein. Also, based on recent results from our investigations, we propose an alternative mechanism of D1 degradation where a stromal protease(s) participates in the degradation.

Mechanism of photodamage to the D1 protein

Photoinhibition of photosystem II and turnover of the photodamaged D1 protein have been studied both in vivo and in vitro. Proposed molecular mechanisms of photoinhibition and D1 protein turnover are derived mostly from studies in vitro. Two mechanisms have been proposed describing the photodamage to the D1 protein, namely donor-side and acceptor-side photoinhibition of photosystem II (Barber and Andersson 1992, Aro et al. 1993). As these two mechanisms nicely explain the phenomena observed in vitro, it is believed that these mechanisms are functioning in vivo.

In the acceptor-side photoinhibition model, damage to the D1 protein is thought to be caused by an over-reduction of plastoquinones at the acceptor-side of photosystem II. To induce acceptor-side photoinhibition, we usually illuminate thylakoids or photosystem II preparations, which retain water oxidation activity, with strong light. Under these conditions, double reduction of the plastoquinone electron acceptor Q_A and dissociation from its binding site take place, thereby increasing the probability for charge recombination between the oxidized form of the chlorophyll primary electron donor P680 and the reduced form of the primary electron acceptor pheophytin. This, in turn, leads to an enhancement of the formation of P680 triplet states (Vass et al. 1992), which are able to react with molecular oxygen to produce singlet oxygen, a potentially very damaging species to proteins. It is believed that the formation of singlet oxygen in the immediate vicinity of the D1 protein is the event that initiates the damage and degradation of the D1 protein. Singlet oxygen was detected from photosystem II reaction center preparations by steady-state and time-resolved luminescence measurements (Macpherson et al. 1993). Singlet oxygen formation was also demonstrated by histidine-dependent oxygen uptake and bleaching of *p*-nitrosodimethylaniline by illuminated photosystem II reaction centers; other than singlet oxygen, no oxygen radicals were detected (Telfer et al. 1994). The site of damage is suggested to be on the stroma-exposed DE-loop of the D1 protein. Damage by the singlet oxygen results in the cleavage of the D1 protein generating a 23 kDa N-terminal fragment and a 10 kDa C-terminal fragment (De Las Rivas et al. 1992). Degradation of the D1 protein is not confined to high light conditions. Under low light, it was proposed that back electron flow from Q_B^- or Q_A^- to the S_2 or S_3 state of the donor side of photosystem II may cause oxidative damage to the D1 protein via generation of active oxygen species (Keren et al. 1997).

An alternative interpretation of acceptor-side photoinhibition involves the interaction between the plastoquinone electron acceptor Q_B and molecular oxygen (Kyle et al. 1984). This interpretation has reduced Q_B oxidized by O_2 to form superoxide anion radicals, which is another type of reactive oxygen species potentially harmful to proteins. However, no clear evidence has been obtained supporting the generation of superoxide radicals in the acceptor-side photoinhibition mechanism.

During the degradation of the D1 protein under illumination, conformational changes in the D1 protein around the Q_B binding site also has been indicated as part of the acceptor-side photoinhibition mechanism. The evidence that herbicides that interact with the Q_B-binding pocket suppress light-induced degradation of the D1 protein supports this idea. Furthermore, this idea is supported by the finding that the binding of the Q_B inhibitor *N*-octyl-3-nitro-2,4,6-trihydroxy-benzamide (PNO8) triggers the specific cleavage of the D1 protein into 23-kDa N-terminal and 9-kDa C-terminal fragments in the dark, which is enhanced upon preillumination with weak light (Nakajima et al. 1995, Nakajima et al. 1996). Thus, it appears that certain protein structural changes are required for the efficient degradation of the D1 protein.

In the donor-side photoinhibition model, it is proposed that the degradation of the D1 protein is mediated through long-lived and highly oxidative species, such as $P680^+$ and the oxidized tyrosine electron donor Tyr Z^+ (Jegerschöld et al. 1990). These species are long-lived when the photosystem II complexes loses the catalytic Mn cluster or when the final O_2-evolving step is inhibited, for example, under low chloride conditions. Donor-side photoinhibition is easily demonstrated with an in vitro system, because it has a larger quantum yield than acceptor-side photoinhibition and occurs even under weak illumination. In our experiments, we usually use spinach photosystem II membrane preparations that are treated with Tris (0.8 M, pH 9.0) to remove the OEC subunits and the Mn-cluster to inhibit water oxidation. In some cases, Mn-cluster and part of

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the OEC subunits were removed from photosystem II membranes by hydroxylamine treatment (3 mM, pH 6.5).

In a series of studies by Cheniae and coworkers with hydroxylamine-treated photosystem II membranes, the site of photodamage and the mechanism of donor-side photoinhibition was described concretely. During weak light photoinhibition, two sites of photodamage were detected at the donor side of photosystem II; these sites are Tyr_Z and possibly Tyr_D (Blubaugh and Cheniae 1990). Following with EPR and optical spectrophotometric analyses, the order of susceptibility of photosystem II components to photodamage in donor-side photoinhibition was suggested as chlorophylls / carotenoids > Tyr_Z > Tyr_D >> P680, Pheophytin, Q_A (Blubaugh et al. 1991). Under strict anaerobic conditions, photoinactivation of photosystem II was greatly inhibited even in the presence of an electron acceptor, and participation of superoxide radicals in photodamage was suggested (Chen et al. 1992, Chen et al. 1995). Although oxygen-independent degradation of the D1 protein has been usually taken as a typical feature of the donor-side photoinhibition of photosystem II (Jegerschöld et al. 1990), participation of oxygen radicals in the donor-side mechanism of degradation of the D1 protein is not excluded. Thus, the role of oxygen in the turnover of the D1 protein requires further investigation.

Data obtained on the induction of donor-side photoinhibition from in vivo studies were often related to the data gained from in vitro studies. For example, the in vivo degradation pattern of the D1 protein from light-stressed pumpkin leaves was similar to that observed in vitro in the donor-side mechanism; thus indicating that donor-side photoinhibition occurs in vivo (Kettunen et al. 1996). However, the primary cause of the donor-side photoinhibition under natural conditions is not yet clear. Low pH in the thylakoid lumen induced by light, depletion of Cl^-, and low temperature might be the cause of donor-side photoinhibition. A model proposed by Anderson et al. (1998) based on in vivo studies shows that only one photon is required for the photodamage to photosystem II and that the primary cause of damage to the D1 protein is $P680^+$, rather than singlet oxygen, which suggests a major role of donor-side photoinhibition under natural conditions.

In donor-side photoinhibition, damage occurs at the lumen-exposed AB loop of the D1 protein, producing two fragments by cleaving the protein into a 10 kDa N-terminal portion and a 24 kDa C-terminal portion (De Las Rivas et al. 1992). Other fragments having apparent molecular masses of 16–18 kDa were also observed in vitro, which were attributed to the cleavage of the D1 protein in the lumenal loop connecting helices C and D (Shipton and Barber 1991, Barbato et al. 1992d). Regardless of the exact mechanisms involved in the photodamage in donor-side and acceptor-side photoinhibition, the process of degradation of the D1 protein is complicated by the action of specific or non-specific proteases, as described below.

Degradation of the D1 protein—a direct pathway

After the D1 protein is photodamaged, the protein is degraded by proteases. Protease activities responsible for the degradation of the D1 protein have been shown to degrade the D1 protein directly (the direct pathway of D1 degradation). Western blot experiments with specific antibodies against the D1 protein show the results of protease action by the appearance of D1 fragments at molecular mass ranges less than that for the native D1 protein. It is not known, however, whether the photodamaged D1 protein is digested by D1-specific proteases, by general house-keeping proteases, or both.

As chloroplasts have a prokaryotic origin, it is assumed that proteases homologous to those found in prokaryotes are functioning in the degradation of structurally unfavorable proteins within chloroplasts (Adam 1996). In fact, proteases having homology with a cytoplasmic protease ClpP, a membrane-bound protease FtsH, and a periplasmic protease DegP in *Escherichia coli* were identified in the stroma, thylakoid membranes, and the thylakoid lumen of chloroplasts, respectively (Shanklin et al. 1995, Lindahl et al. 1996, Itzhaki et al. 1998). ClpP is a serine-type protease with Ser, His and Asp in the active center, and forms a homooligomer. The homooligomeric ClpP is associated with ATPase subunits which also form a homooligomer, and they finally show a barrel-like structure. The stromal ClpP was shown to be involved in the degradation of cytochrome b_6/f complex (Majeran et al. 2000). FtsH is a membrane-bound zinc metalloprotease with two trans-membrane helices and a large hydrophilic domain with an ATP binding site and a catalytic site. The thylakoid FtsH protease is known as an AAA (ATPases associated with a variety of cellular activities) protease. The means of proteolysis of integral membrane proteins by AAA proteases has been proposed recently (Langer 2000). One possibility is that AAA proteases cleave loops or domains exposed from membrane-embedded polypeptides. Another possibility is that AAA proteases cleave membrane-embedded polypeptides. They extract the membrane-embedded portions from the lipid bilayer and mediate protein degradation in a hydrophilic environment. FtsH was shown to degrade an unassembled Rieske Fes protein (Ostersetzer and Adam 1997). FtsH is also involved in the assembly of functional photosystem I in cyanobacteria (Mann et al. 2000).

Recent works by Spetea et al. (1999) and Lindahl et al. (2000) support the direct digestion of the photodamaged D1 protein. They suggested a direct proteolytic cleavage of the D1 protein to produce a 23-kDa N-terminal fragment, observed to be GTP-dependent, which is then degraded by the thylakoid FtsH protease.

Degradation of photosystem II polypeptides other than the D1 protein under light stress

While the D1 protein is the most photosensitive protein in photosystem II, other photosystem II proteins also undergo degradation during strong illumination. In particular, the D2 protein, which is the D1 counterpart of the reaction center het-

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erodimer, is degraded in parallel with the D1 protein, but to a lesser extent (Schuster et al. 1988, Barbato et al. 1992a). During donor-side photoinhibition of photosystem II-enriched membranes induced by strong illumination, the antenna-chlorophyll-binding protein CP43 also is degraded (Yamamoto and Akasaka 1995). Since CP43 is not directly involved in the electron transport of photosystem II, the observed degradation may be due to its location in the vicinity of the long-lived strong oxidants created in photosystem II under light stress. Also, light-induced degradation of cytochrome b_{559} was reported recently (Ortega et al. 1999). Putative proteases responsible for the degradation of these proteins have not been identified yet.

Conformational changes of photosystem II during photoinhibition and related processes

Conformational changes of photosystem II and also of the polypeptides comprising photosystem II have been investigated during photoinhibition. Conversion of dimeric photosystem II complexes to the monomeric form during photoinhibition was shown by comparing the elution patterns in size exclusion HPLC of photosystem II complexes before and after photoinhibitory illumination (Kruse et al. 1997). By contrast, no change to the overall size of photosystem II complexes after photoinhibition was detected by electron microscopy and single particle analysis (Kitmitto et al. 1999). Measurements with Fourie transform infrared (FT-IR) spectroscopy after strong illumination of non-oxygen-evolving photosystem II reaction center samples showed changes in the content of α-helix and β-sheet structures by as much as 30%, which occurred in parallel with degradation of the D1 protein (He et al. 1991). Later studies with FT-IR lead to conflicting results. After prolonged illumination of photosystem II membranes, no clear change was observed in the structure of photosystem II (Ono et al. 1995). In another study, a large decrease in α-helix content and a parallel increase in β-sheet content were observed after strong illumination of photosystem II core samples (Zhang et al. 1997). In experiments with Tris- or hydroxylamine-treated photosystem II core complexes, it was shown that a larger change is detectable in the signals of FT-IR of photosystem II samples devoid of OEC subunits, compared with samples retaining OEC subunits (Yamamoto et al. 1998).

It has been proposed that photosystem II complexes containing photodamaged D1 proteins migrate from the thylakoid grana-stacks to the stroma-exposed regions of the thylakoids for repair (Hundal et al. 1990, Barbato et al. 1992c). As CP43 is relatively easily liberated from the photosystem II complexes during photoinhibition (Barbato et al. 1992c), it is possible that CP43 acts as a carrier of the damaged D1 protein. Formation of observed D1/CP43 cross-linked products (see following section) might be related to this putative function of CP43. Cross-linking of the D1 protein to CP43 was affected by protein phosphorylation (Miyake and Yamamoto, unpublished data). It is possible that phosphorylation of the D1 adducts stimulates migration of the adducts from the granal thylakoids to the stromal thylakoids. These results should be examined further based on the proposed mechanism that phosphorylation of the D1 protein regulates the rate of degradation of the D1 protein (Koivuniemi et al. 1995, Rintamäki et al. 1996), and also on recent results that contradict this mechanism (Mizusawa et al. 1999).

Cross-linking of the D1 protein with the surrounding polypeptides in photosystem II

In the course investigating photoinhibition, we found that cross-linking of the D1 protein with the surrounding polypeptides takes place during illumination of photosystem II-enriched membranes under aerobic conditions (Mori et al. 1995, Yamamoto and Akasaka 1995, Yamamoto et al. 1998, Ishikawa et al. 1999). The term “cross-linking” in this review describes both the aggregation or covalent adduct-formation of the photosystem II components, although the exact nature of the binding is not known. Cross-linking of the D1 protein with CP43 was first indicated by a decrease in the amount of Coomassie-stained CP43 band following SDS/urea-polyacrylamide gel electrophoresis after strong illumination of photosystem II membranes (Mori and Yamamoto 1992). Western blot analysis with antibodies against both the D1 protein and CP43 showed that the D1 protein forms cross-linked products with CP43 within a molecular weight range of 70–100 kDa (Mori et al. 1995, Yamamoto and Akasaka 1995) (Fig. 2). In addition to the D1/CP43 adduct, a 41 kDa cross-linked product between the D1 protein and the α-subunit of cytochrome b_{559} (Barbato et al. 1992b), and a D1/D2 heterodimer are generated by illumination of the photosystem II under aerobic conditions. Among these adducts, the 41-kDa cross-linked product of the D1 protein and the α-subunit of cytochrome b_{559} is the best characterized. Cross-linking was shown to take place between the N-terminal serine of the α-subunit of cytochrome b_{559} and the 239Phe-244Glu region in the DE loop of the D1 protein (Barbato et al. 1995). In a more recent study, however, mutants of *Synechocystis* sp. PCC6803 having deletions in the DE loop of the D1 protein indicated an alternative D1 cross-linking site (Barbato et al. 1999). Additionally, the cross-linking between the D1 protein and the α-subunit of cytochrome b_{559}, and that between the D1 protein and D2 protein, were shown to depend on the presence of oxygen, which suggests a role of reactive oxygen in the cross-linking process. The sensitivity of the cross-linked products to a stromal protease(s), discussed in the next section, suggests that these cross-linked sites are exposed to the stromal side of the proteins (Ishikawa et al. 1999). Cross-linking of the D1 protein was observed not only in photosystem II-enriched membranes, but also in thylakoids and intact chloroplasts (Ishikawa et al. 1999).

Among the three types of D1 adducts, the D1/D2 heterodimer is most easily generated by the illumination, followed by the cross-linked product between the D1 protein and

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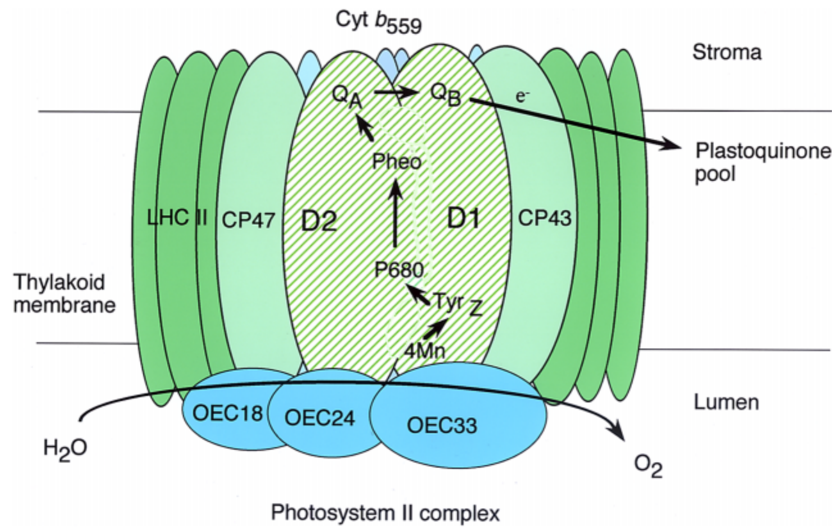

Fig. 1 A schematic diagram showing the structure of the photosystem II complex. The relative locations of the D1 protein, CP43 and OEC33 are shown based on results in the cited references (Mori et al. 1995, Yamamoto and Akasaka 1995, Yamamoto et al. 1998).

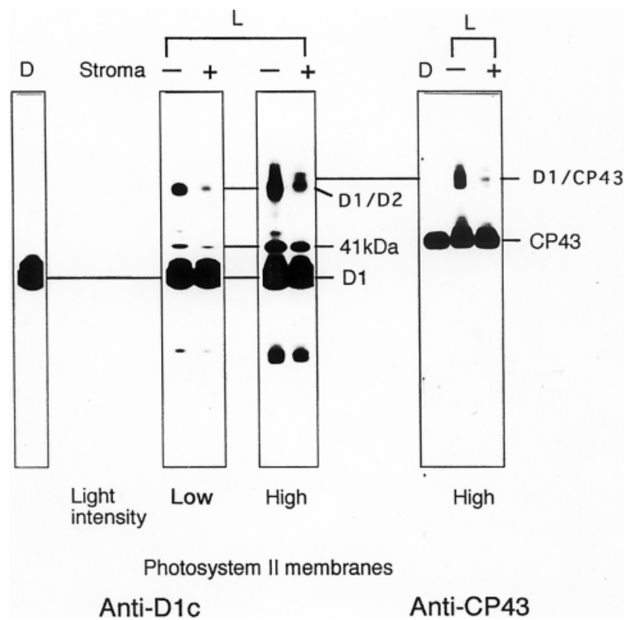

Fig. 2 A fluorogram showing the photo-induced cross-linking of the D1 protein and the digestion of the cross-linked products by a stromal protease(s). Spinach photosystem II membranes were illuminated either with low light (50 µE m^{-2} s^{-1}) or with high light (5,000 µE m^{-2} s^{-1}) for 50 min under aerobic conditions, and then incubated in darkness, for 20 min at 25°C, with (+) or without (–) added stromal fractions prepared from intact chloroplasts. D and L denote the dark-control and illuminated samples, respectively. After SDS/urea-polyacrylamide gel electrophoresis and Western blotting with specific polyclonal antibodies against the C-terminal part of the D1 protein and CP43 (indicated at the bottom of the gels), the native D1 and the cross-linked products of D1 (shown at the right of the gels) were detected by enhanced chemiluminescence (ECL).

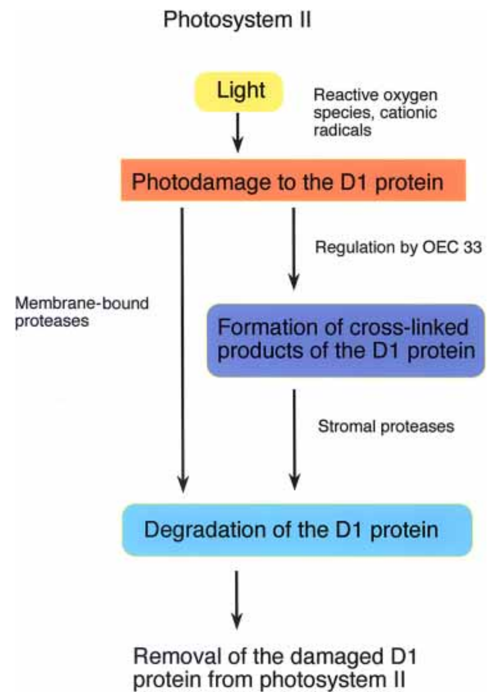

Fig. 3 A diagram showing the pathways of degradation of the photo-damaged D1 protein.

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the α-subunit of cytochrome b_{559}, and the D1/CP43 adduct. Illumination of samples at light intensity of 500 μE m^{-2} s^{-1} for 60 min is sufficient to generate the D1/D2 heterodimer and D1/the α-subunit of cytochrome b_{559} cross-linked products, whereas a stronger light, such as 2,000–5,000 μE m^{-2} s^{-1}, is required for the generation of the D1/CP43 adducts. The cross-linked products of the D1 protein appear more prominently compared to the D1 fragments alone, suggesting that the D1 cross-linking process is a sensitive indicator of the photodamage to the D1 protein. However, the relative levels of these illumination-dependent cross-linked products, as well as the cross-linking sites on the polypeptides, remain to be determined.

Cross-linking of the photosystem II polypeptides is likely due to oxidation of amino acids in the D1 protein that is followed by a conformational change of the protein. Investigation using mass spectroscopy showed the oxidation of amino acids in the D1 and D2 proteins to be distributed around the photoreactive components of photosystem II (Sharma et al. 1997). This oxidation might initiate the subsequent covalent and non-covalent association of the proteins. Cross-linking of the D1 protein with the D2 protein, with the α-subunit of cytochrome b_{559}, and with CP43 indicate that these proteins are located in close proximity to each other. Cross-linking of the D1 protein and CP43 especially supports the recent structural model of photosystem II complex, showing adjacent positioning of these proteins as diagrammed in Fig. 1 (Barber et al. 1999). As another example of D1 cross-linking, a 160 kDa cross-linked product was reported by the illumination of *Dunaliella salina* (Baroli and Melis 1996).

Digestion of the cross-linked products of the D1 protein by a stromal protease(s)—a new proposal for the degradation pathway of the photodamaged D1 protein

The cross-linked products of the damaged D1 protein are processed by proteases located in the stroma of chloroplasts. When pre-illuminated photosystem II samples were incubated with stromal fractions isolated from intact chloroplasts, cross-linked products of the D1 protein decreased significantly (Ishikawa et al. 1999) (Fig. 2). The stromal-extract-dependent decreases were observed with each form of the D1 cross-linked products, namely D1/the α-subunit of cytochrome b_{559}, D1/D2, and D1/CP43. Thus, the D1 cross-linked products are digested by a putative stromal protease(s). The stromal protease(s) is a serine-type protease, which is also SDS-stable. The protease(s) has a pH optimum of 8.0, and requires ATP or GTP (Ferjani et al. 2001). Two-dimensional SDS/urea-polyacrylamide gel electrophoresis showed the presence of a 15-kDa SDS-stable protease from the stroma, which digested the D1 cross-linked products (Ishikawa et al. 1999). Several SDS-stable proteases have been reported with spinach chloroplasts; of which, three proteases having apparent molecular masses of 14, 30 and 54 kDa, are present in stromal extracts (Sokolenko et al. 1997). At present, it is not known whether the 15-kDa stromal protease described above is the same as the SDS-stable 14-kDa stromal protease identified by Sokolenko et al. (1997). Based on the observed results, we propose a new pathway for degradation of the photodamaged D1 protein (Fig. 3). In this model, photodamaged D1 protein cross-links with the D2 protein, the α-subunit of cytochrome b_{559}, and CP43, and these cross-linked products are digested by a stromal protease(s).

Regulation of the degradation of the D1 protein by OEC 33 of photosystem II in donor-side photoinhibition

The OEC subunits of photosystem II are present in the thylakoid lumen as membrane-bound and free forms. Ettinger and Theg (1991) first discovered relatively large pools of free OEC subunits in the thylakoid lumen from pea and spinach. The OEC subunits were shown to have long lifetimes, exceeding 8 h in vitro, and were competent for assembly into the membrane-bound complex (Hashimoto et al. 1996). In the process of assembly of the OEC subunits into photosystem II, it was suggested that each subunit binds to the core complex of photosystem II in a stepwise manner; OEC33 binds to photosystem II in the stromal-exposed membranes, and then OEC24, and likely OEC18, bind to photosystem II in the thylakoid grana membrane regions (Hashimoto et al. 1997). Although the binding of the OEC subunits is essential for oxygen evolution activity in photosystem II, the OEC subunits are liberated from photosystem II concurrently with the degradation of the D1 protein when photosystem II-enriched samples are irradiated with strong light (Hundal et al. 1990). The timing of degradation of the D1 protein and the release of the OEC subunits is likely critical for the efficient turnover of the D1 protein. When the photosystem II membranes lacking OEC subunits are illuminated, significant cross-linking of the D1 protein and the surrounding polypeptides takes place due to donor-side photoinhibition (Yamamoto and Akasaka 1995). From a reconstitution study, it was suggested that OEC33 prevents the D1 protein from cross-linking and thereby stimulates the degradation of the D1 protein (Yamamoto et al. 1998). Thus, in donor-side photoinhibition, OEC33 seems to play a crucial role in maintaining the structure of the photosystem II complex when the D1 protein is photodamaged and subsequently degraded (Fig. 3). It is possible that OEC 33 works like a molecular chaperone and prevents the photodamaged D1 protein from non-specific aggregation at the donor-side of photosystem II.

The aggregates of the D1 protein formed at the stromal-exposed side of photosystem II are degraded by a stromal protease(s). Our concern is whether a protease(s), which recognizes the photodamaged D1 protein, also is located in the chloroplast thylakoid lumen. Recently, a large number of polypeptides in the thylakoid lumen were well characterized by two-dimensional electrophoresis, mass spectroscopy and protein sequencing (Kieselbach et al. 1988, Peltier et al. 2000). In the near future, a protease from the thylakoid lumen that digests the photodamaged D1 protein may be identified. If the aggregates of the D1 protein and CP43 formed at the lumenal side of photosystem II in donor-side photoinhibition are not

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digested by a lumenal protease(s), then the only way to avoid the protein aggregation at the donor-side of photosystem II may be protection of the D1 protein by OEC33. It was also suggested that OEC33 is a thermostable and exists naturally in an unfolded state (Lydakis-Simantiris et al. 1999). Plus, the OEC subunits are rich in β-sheet content, which contributes to an extended structure (Xu et al. 1994, Zhang et al. 1998, Zhang et al. 1999). Also, taking into account the long life-time of the protein even in the non-bound form (Hashimoto et al. 1996), we assume that OEC33 is suitable to protect the D1 protein from photodamage and cross-linking. The extended structure of OEC33 may shield a relatively large surface of the lumen-exposed loops of the D1 protein, which is the possible cross-linking site, and may protect the D1 protein from the action of oxygen radicals formed in the light. The natively unfolded structure of OEC33 also may be necessary for the recognition of newly organized photosystem II complexes and efficient binding of OEC33 from the lumenal pool during the turnover of the D1 protein. Moreover, the thermostability of OEC33 might help to protect the D1 protein from heat stress as well as from light stress. These possibilities should be examined by future experiments.

Concluding remarks

It has become increasingly apparent that the proteases in chloroplasts play a pivotal role in the quality control of photosystem II. The proteases responsible for the degradation of photodamaged D1 protein have not been determined yet, and we must await further progress in the identification and characterization of these proteases to have a better understanding of the nature of the photo-induced D1 degradation process. It is probable that various proteases along with other components cooperate to accomplish the efficient turnover of the D1 protein. Protein aggregation is a general phenomenon observed during oxidative stress in cells. Photodamage and cross-linking of the D1 protein is a typical occurrence taking place through photooxidative processes during illumination of chloroplasts. If these cross-linked products were allowed to accumulate, then lethal effects on the chloroplasts might be imposed. We propose a model showing that a proteolytic system works in the stroma to remove the cross-linked products of the photodamaged D1 protein. A 33-kDa subunit of the oxygen-evolving complex (OEC) bound to the lumen side of photosystem II regulates the formation of the cross-linked products in donor-side photoinhibition. These functionally inter-related systems may enable the efficient turnover of the D1 protein during the light stress to chloroplasts.

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