*J. Biosci.,* Vol. 20, Number 1, January 1995. pp 35–47. © Printed in India.

# D1 protein of photosystem II: The light sensor in chloroplasts

U DWIVEDI and R BHARDWAJ*
School of Biochemistry, D A University, Khandwa Road, Indore 452 001,India

MS received 24 May 1994; revised 5 November 1994

**Abstract.** Light, controls the "blueprint" for chloroplast development, but at high intensities is toxic to the chloroplast. Excessive light intensities inhibit primarily photosystem II electron transport. This results in generation of toxic singlet oxygen due to impairment of electron transport on the acceptor side between pheophytin and $Q_B$ -the secondary electron acceptor. High light stress also impairs electron transport on the donor side of photosystem II generating highly oxidizing species $Z^+$ and $P680^+$. A conformationsl change in the photosystem II reaction centre protein Dl affecting its $Q_B$-binding site is involved in turning the damaged protein into a substrate for proteolysis.

The evidence indicates that the degradation of D1 is an enzymatic process and the protease that degrades D1 protein has been shown to be a serine protease Although there is evidence to indicate that the chlorophyll *a*–protein complex CP43 acts as a serine-type protease degrading Dl, the observed degradation of Dl protein in photosystem II reaction centre particles *in vitro* argues against the involvement of CP43 in Dl degradation. Besides the degradation during high light stress of Dl, and to a lesser extent D2-the other reaction centre protein, CP43 and CP29 have also been shown to undergo degradation.

In an oxygenic environment, Dl is cleaved from its N- and C-termini and the disassembly of the photosystem II complex involves simultaneous release of manganese and three extrinsic proteins involved in oxygen evolution. It is known that protein with PEST sequences are subject to degradation; D1 protein contains a PEST sequence adjacent to the site of cleavage on the outer side of thylakoid membrane between helices IV and V.

The molecular processes of "triggering" of Dl for proteolytic degradation are not clearly understood. The changes in structural organization of photosystem II due to generation of oxy-radicals and other highly oxidizing species have also not been resolved. Whether CP43 or a component of the photosystem II reaction centre itself (Dl. D2 or cy1 b559 subunits), which may be responsible for degradation of Dl, is also subject to light modification to become an active protease, is also not known. The identity of proteases degrading Dl, LHCII and CP43 and C29 remains to be established.

**Keywords,** Photosystem II; Dl protein turnover; photoinhibition; chloroplast.

## 1. Light interaction in photosynthetic organisms

Light interception is dependent on the disposition of the photosynthetic tissues related to incoming radiations. In an individual canopy, the photosynthetic tissues orient themselves to maximize interaction with light, but in natural environments the quantity of light received by the leaves is rarely constant even at the top of the canopy.

The simultaneous operation of two photosystems for light dependent cleavage of water and NADP reduction and the fact that the quantum yield of photosynthesis

*Corresponding author.

is constant under varying light regimes would appear to require that both photosystems have an equal distribution of chlorophyll (chl). Inspite of this requirement, the distribution of chl between the two photosystems is uneven; photosystem II (PS II) has larger light harvesting antenna than that of photosystem I (PS I) (Anderson and Osmond 1987). This imbalance in the distribution of chl is more pronounced in plants growing under conditions of shade (shade adapted species). Shade adapted species are characterized by extensive grana stacks and the degree of stacking is reflection of an uneven distribution of chl between the two photosystems. This stacking could be the result of an excess of the light harvesting chl *a*/*b* –protein complex (LHC II) in shade plants; LHC II may not be functionally associated with any of the photosystems and thus its primary role may be stacking the thylakoids. Grana stacking is one of the intriguing features of thylakoid structure whose functions are not yet clearly understood [stacking does not appear to be a device for increasing interception of light since *in vitro* experiments suggest that grana stacks absorb less light than the unstacked membranes (Jennings and Zucchelli 1985) ] .

When irradiance is high for a brief period, the attainment of the maximum quantum yield requires that PS II and PS I are excited at appropriate rates. This is achieved atleast in part by reversible phosphorylation of small population of the peripheral LHC II of the PS $II_{0C}$ centres that adjusts the relative cross-sections of the photosystems (see Dwivedi and Bhardwaj 1994). The phosphorylation-linked migration of LHC II from the appressed regions of the membrane is known to cause partial destacking as well as increase in PS I photochemistry. Since the PS II is sluggish (low quantum yield) compared to PS I, the coupling of phosphorylated-LHC II with PS I may be to use "sunflecks" and consequently increase cyclic photophosphorylation.

In the short term, the quantum yield of PS II is regulated by the non-photochemical quenching of excitation energy induced by a high transthylakoid Δ pH. This major regulatory response allows harmless dissipation of excess excitation energy as heat in the PS II antenna (Horton 1989). It is of fundamental importance for the protection of the photosynthetic apparatus against photo-destruction. Excess excitation energy must be dissipated to prevent over-reduction of the electron transport components.

Prolonged exposure to light levels where excitation energy exceeds the capacity for carbon assimilation can lead to photoinhibition. Photoinhibition is defined as the reduction in photosynthetic activity by excessive light and is evidenced by loss of quantum efficiency and changes in chl fluorescence characteristics (Powles 1984; Barber and Andersson 1992; Virgin *et al* 1992; Aro *et al* 1993).

Much progress has been made in understanding the molecular architecture of PS II as well as light dependent turnover of the proteins of PS II complex. This review attempts to cover the events that trigger photoinactivation of PS II electron transport during high light stress and the subsequent degradation of Dl protein. An attempt has also been made to highlight areas which need to be explored further.

## 2. The PS II complex

PS Π is a supra-molecular complex made up at atleast 25 proteins (see figure 1). A number of proteins bind chl, forming a light harvesting antenna. The smallest PS Π reaction centre complex which binds the reaction centre pigment P680, the

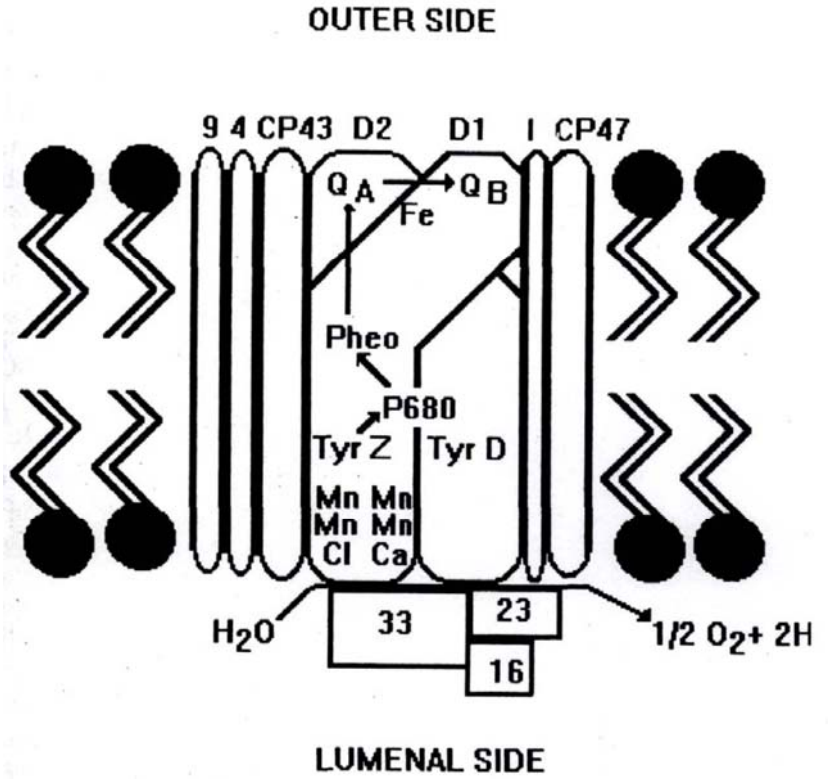


**Figure 1.** A schematic simplified presentation of the subunit structure of PS II. For simplicity LHC-II and other polypeptides are not shown. The PS II reaction centre consists of heterodimeric Dl, D2 proteins and two subunits of cyt $b_{599}$ The redox components involved in charge separation and stabilization are bound to heterodimeric D1/D2. 'Z' is Tyr-161 in Dl polypeptide. The heterodimeric reaction centre binds 4–5 chl *a* and two pheophytin and a non-heme iron. Tyr is counter part of Tyrz in the side path. The secondary electron acceptors $Q_A$ and $Q_B$ are bound to Dl and D2 polypeptides. respectively. The two light harvesting chlorophyll $\alpha$-protein complexes CP43 and CP47 are also shown. Four Mn atoms forming clusture and probably bound to Dl polypeptide constitute the charge storage catalyst. The other two co-factors $Ca^{2+}$ and $Cl^{-}$ also interact on one side with Mn clusture and stabilize three oxygen evolving extrinsic proteins on the lumenal side Three extrinsic proteins involved in oxygen evolution are on the lumenal side while all other proteins are transmembrane proteins in the lipid bilayer. " I " is the product of *psbI* gene with a molecular mass of 4·5 kDa. The numbers indicate the molecular masses of the polypeptides. Adapted from the information existing in the literature.

primary acceptor pheophytin as well as the secondary acceptors $Q_A$ and $Q_B$. consists of Dl, D2 and the 9 and 4 kDa subunits of cyt *b* (Nanba and Satoh 1987; Barber *et al* 1987; Marder *et al* 1987). The heterodimeric D1-D2 proteins together bind four chl $\alpha$ and two pheophytin molecules. Dl and D2 are thus apoproteins of PS II reaction centre (Mattoo *et al* 1989).

The PS II core complex is composed of two major chl $\alpha$ binding antenna proteins CP47 and CP43 which are coded by the chloroplast *psbB* and *psbC* genes, respectively (figure 1). CP29 (Barber and Andersson 1992) and the 22 kDa intrinsic protein

shown to be a chl binding protein by Funk *et al* (1994) recently, form minor components of the light harvesting antenna.

PS II particles in addition to the reaction centre components, also contain three additional proteins-34, 23 and 16 kDa which are involved in oxygen evolution (Kuwabara and Murata 1982; Satoh *et al* 1983; Kuwabara *et al* 1985; Tang and Satoh 1985).

## 3. The PS II reaction centre Dl protein

### 3.1 *Synthesis of D1 protein*

The *psbA* gene, located on chloroplast genome and coding for Dl protein, is in most cases as a single uninterrupted copy in the large single copy (LSC) region near the inverted repeat. The gene location and nucleotide sequence of the gene in different species is highly conserved (< 90%) (Zurawski *et al* 1982; see also Trivedi *et al* 1994). The *psbA* gene is under the control of bacterial like promotor consensus sequences (–10/-35 sequences). The *psbA* promoter is a strongly expressed promoter (Gruissem and Zurawski 1985): any point mutation results in a *down* promoter mutation.

3.1a *Light regulation of Dl synthesis*: Gene transcripts increase substantially during etioplast transformation in light (Link 1982; Fromm *et al* 1985). The transcripts are quite stable in the stroma and their level has not been found to be altered during light-dark transitions. The transcript is probably stabilized by nuclear-coded protein(s) involving 3' inverted repeats of the mRNA (see Aro *et al* 1993).

Light is essential for the translation of the mRNA (Fromm *et al* 1985). Thus, the expression of *psbA* in algae and higher plants is controlled by light at the post-transcriptional or translational level. *psbA* transcripts have been found in non-polysomal fractions in the stromal phase in dark (Klein *et al* 1988). Thus either there may be a block in translation in dark or light promotes engagement of the transcripts into the polysomal fraction. A nuclear-coded 47 kDa protein has been identified to activate translation of the mRNA involving its binding to 5' end in light (see Aro *et al* 1993). The synthesis of complete Dl protein and its stabilization is also under control of transformation of protochlorophyllide to chl in light. The binding of chl to the growing polypeptide chain helps in completion and maturation of Dl protein (Mullet *et al* 1990). No Dl protein synthesis occurs in darkness (see Aro *et al* 1993).

Although there are apparently two initiation codons (met-1 and met-37), it has conclusively been proved that initiation begins at met-1 codon (Eyal *et al* 1987; Michel *et al* 1988) synthesizing a precursor which shows an apparent molecular mass of 33·5 to 34·5 kDa on SDS-PAGE, though from the number of codons in Dl transcript, a 39 kDa polypeptide is predicted (if transcription begins at met-1) (Zurawski *et al* 1982). The precursor is post-translationally processed from its C-terminal end (Marder *et al* 1984; Takahashi *et al* 1988) by probably a loosely bound thylakoid processing enzyme of 34 kDa (Inagaki *et al* 1989) after insertion of the precursor into the thylakoids.

Pulse chase experiments established that Dl protein is initially inserted into stromal lamellae and processed from C-terminal ala 344; the processed Dl protein

is then translocated from stroma to grana lamellae. The signal for translocation of processed D1 to the grana region is not known but the translocation is probably regulated by light-induced conformational change. The translocation of the processed Dl protein could probably be coupled to the increase in its hydrophobicity. Alternatively, stromal membranes being rich in proteins of PS I complex (and consequently more negatively charged stromal lamellae), processed Dl protein may be excluded from stromal region leading to integration of Dl protein in the appressed regions of the thylakoids.

The synthesis and turnover of Dl protein is regulated by light (Mattoo *et al* 1984). At low light (30 μE $m^{-2}$ $s^{-1}$) under steady state conditions, the concentration of Dl protein is 85% and 15% in the grana and stroma, respectively (Callahan *et al* 1990). The half life of Dl protein is dependent on light intensity.

### 3.2 *The structure of D1 protein*

The deduced amino acid sequence of the *psbA* gene predicted a hydrophobic nature for Dl protein (Zurawski *et al* 1982) with five transmembrane helices (Trebst 1987). Besides five membrane spanning helices, the protein forms one CD helix (between helices III and IV) on the lumenal side and another DE helix (between helices IV and V) on the stromal side. The electron donor to the oxidized P680 Z is a tyr-161 radical of the Dl protein (Barry and Babcook 1987). The helices IV ahd V are connected to each other by an Fe atom bridged *via* two histidine residues (His-215 and His-272) (figure 2). Dl protein is also connected to D2 protein through this Fe atom involving two other histidine bridges. This area is the $Q_B$ site where plastoquinone (PQ) binds during electron transfer.

The herbicide and $Q_B$ binding niche sites subject to mutations on Dl protein lie in the transmembrane helices IV and V and in the hydrophobic region between helices IV and V on the matrix side (Trebst 1987). All mapped mutations in Dl protein fall between amino acid residues 211 to 275. The mutation in this area does not affect $Q_B$ binding. The amino acids modified by photoaffinity labelling (Oettmeier *et al* 1980: Dostatni *et al* 1988) could well be those that can not be mutated without loss of $Q_B$ function.

### 3.3 *The light dependent photoinactivation of PS II electron transport*

The PS II is primary target of attack when plants are exposed to excessive light intensities (Barber and Andersson 1992; Salter *et al* 1992; Aro *et al* 1993). Impairment of PS II electron transport leads to photoinactivation. The impairment of PS II electron transport under excessive light intensities (more than encountered during growth) has been shown to be due to a functional impairment of $Q_A$ on the reducing side of PS II or due to accumulation of highly oxidized species on the donor side of the PS II (see Aro *et al* 1993).

Considerable efforts have been made to identify the sites where photoinhibition starts, but the actual mechanism for light induced irreversible damage of the electron transport chain and the triggering event(s) are not clearly understood.

Two main mechanism have been proposed: the first, referred to as the "acceptor side" mechanism, implies impairment at the level of secondary electron acceptors $Q_A$ and/or $Q_B$ (Kyle *et al* 1984; Setlik *et al* 1990; Styring *et al* 1990). The second,

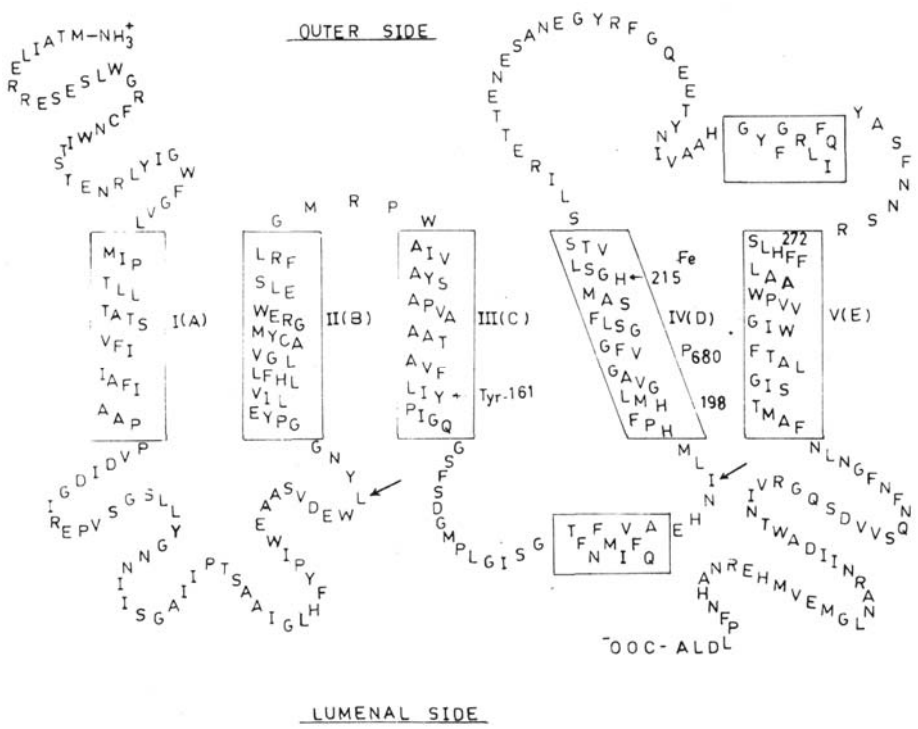


**Figure 2.** Predicted folding pattern of Dl protein of spinach and possible sites of cleavage of Dl protein during light-induced degradation. The Dl protein forms five transmembrane helices marked as I–V (A–E) as well as forms one CD helix on the lumernal side and one DE helix on outer side. The protein sequence has been taken from the literature. Helix D is only expected to be tilted. Z that functions as electron donor to the oxidized P680 is a Tyr-161 (Barry and Babcock 1987). His-198 probably ligates with P680 while His-215 and His-272 stabilize the non-heme iron. The probable sites of cleavage of Dl after photoinactivation are marked with arrows. The model is modified from Barber and Andersson (1992) and is based on Trebst (1987).

referred to as the "donor side" mechanism implies the accumulation of highly oxidizing species such as $Tyr_Z^+$ and/or $P680^+$ (Theg *et al* 1986; Jegerschold *et al* 1990).

3.3a *The acceptor-side mechanism:* Recent experimental evidence suggest that the damage to PS II under photoinhibitory conditions is due to the impairment of PS II electron transport on acceptor side of PS II.

The photoinactivation process during "acceptor side" induced inhibition has been resolved to four sequential stages (see Barber and Andersson 1992; Salter *et al* 1992; Aro *et al* 1993):

(i) Strong illumination leads to over-reduction of PQ which most likely leaves the $Q_B$ site empty resulting in accumulation of an unusually long lived singly reduced $Q_A^-$ with a half-life time of 30 s as compared to few hundred ms for a normally turning over $Q_A$.

(ii) The accumulated $Q_A^-$ is stabilized by protonation, yielding $Q_A^-$-$H^+$. The semi-stable intermediate $Q_A^-$-$H^+$ is dark stable and is characterized by an electron paramagnetic resonance (EPR) signal. Only singly reduced QA is known to give an EPR signal.
(iii) Further transition of $Q_A^-$ is characterized by the loss of EPR signal, characteristic of doubly reduced $Q_A$. This transition of $Q_A^-$ to a more stable state promoted by low pH probably suggests formation of doubly reduced $Q_A$–$H_2$ or Q leaves the binding site at the reaction centre (Styring *et al* 1990; Vass *et al* 1993).
(iv) The final inactivation intermediate, which is the first irreversible one under aerobic conditions, is characterized by non-decaying prompt fluorescence (Fo) and absence of EPR signal. This non-decaying state is suggested to contain reaction centres with an empty $Q_A$ site (see Aro *et al* 1993).

It has been demonstrated that the amount of $Q_A$ lost from the photoinactivated PS II centres during photoinhibitory treatment of PS II core particles co-relates with the proportion of non-decaying centres, supporting the concept that $Q_A$ leaves the reaction centre site (Styring *et al* 1990).

The photoinhibitory damage to PS II particles under aerobic and anaerobic conditions during photoinhibition are quite contrasting but expected. Strong illumination under aerobic conditions leads quickly to irreversible impairment of PS II electron transport and to the subsequent degradation of Dl protein.

However, under anaerobic conditions, the recovery from the first three stages of photoinactivation is almost complete (see Aro *et al* 1993). Diuron has been shown to block the recovery process which suggests that recovery involves the re-establishment of electron transport between $Q_A$ to $Q_B$ (Mattoo *et al* 1984, 1989).

In the absence of a functional $Q_A$, a chl triplet state is formed ($^3$P68O). The formation of chl triplets under photoinhibitory anaerobic conditions has been detected by EPR spectroscopy. Under aerobic conditions, oxygen quenches the triplet state, generating singrefoxygen and other oxygen-free radicals (Asada and Takahashi 1987; Prasil *et al* 1992). The involvement of oxygen-free radicals in Dl degradation has been established using free radical scavengers (Sopory *et al* 1990; Mishra *et al* 1994) (see § 3·4).

3.3b *The donor-side mechanism*: The donor side induced mechanism of photoinactivation of PS II electron transport has been studied using thylakoids or PS II particles pre-treated with hydroxylamine (Blubaugh and Cheniae 1990) or that were chloride-depleted (Jegerschold *et al* 1990) or using site directed mutants in which the donor side ligands were altered (van der Bolt and Vermass 1992).

Donor side inactivation is due to impairment of PS II electron transport between the Mn cluster of the oxygen-evolving complex and P680 (Blubaugh and Cheniae 1990; Jegerschold *et al* 1990). In hydroxylamine treated PS II membranes, three kinetically distinguishable steps have been characterized (Blubaugh and Cheniae 1990); the first step is the decrease in the rate of electron transfer from Z to P680 probably followed by the loss of $Z^+$. The second phase is slow and possibly is related with the loss of $Tyr_D$. In chloride depleted thylakoid membranes, both the inhibition of oxygen evolution and Dl protein degradation is 15–20 times more sensitive. The protective effect of diuron (DCMU) in chloride depleted thylakoid membranes and the inhibition of diphenyl carbazide dependent indophenol (DPC $\rightarrow$DCIP) reduction under high photon flux density (PFD) suggests accumulation of $P680^+$ and/or $Z^+$ which trigger the degradation of Dl protein.

In two site-directed D2 mutants of the cynobacterium *Synechocystis,* the rate of photoinactivation of the PS II electron transport was found to be greater than in the wild type (van der Bolt and Vermaas 1992). Similarly, in another mutant in which the presumed ligand (His-197) to P68O was changed, leading to a decrease in the operating redox mid-point potential of the $P680/P680^+$ couple, the rate of photoinactivation of the PS II electron transport was also greater than in the wild type (van der Bolt and Vermaas 1992). These results have been explained in terms of accumulation of oxidizing $Z_+/P680^+$.

Photoinhibition of photosynthesis appears to be an inevitable consequence of complicated redox photo-chemistry during the electron transfer from Z to PQ (Powles 1984; Andersson and Styring 1991; Barber and Andersson 1992; Prasil *et al* 1992; Salter *et al* 1992; Virgin *et al* 1992; Aro *et al* 1993). Both the synthesis and degradation of the Dl reaction center protein of PS II is regulated by light; but under high PFD, the rate of degradation is a function of light intensity. The restoration of PS II functions requires the degradation and removal of the degraded subunits of Dl protein followed by reinsertion of a fresh copy of Dl protein in the damaged reaction centre.

### 3.4 *The degradation of Dl protein*

Early *in vitro* studies on isolated pea and *Chlamydomonas* thylakoids demonstrated disappearance of 32 kDa-$Q_B$ protein during photoinhibition (Mattoo *et al* 1984; Ohad *et al* 1985). The degradation products were identified as high molecular weight aggregates and these aggregates included the degradation products of Dl protein besides PS II subunits on SDS-PAGE (Schuster *et al* 1989). It was concluded that an intrinsic thylakoid protease is responsible for the D1 degradation after some strong light induced conformational change (Ohad *et al* 1985).

Dl protein degradation has been studied *in vivo* (Kyle 1987; Mattoo *et al* 1989; Prasil *et al* 1992; Salter *et al* 1992; Virgin *et al* 1992). The degradation of Dl polypeptide can also be observed *in vitro* (Andersson and Styring 1991; Barber and Andersson 1992). The degradation of Dl protein has been demonstrated after photoinhibition of isolated thylakoid preparations (Virgin *et al* 1988; Richter *et al* 1990), PS II preparations (Hundal *et al* 1990), PS II core preparations (Virgin *et al* 1990, 1992) and isolated reaction centre particles.

3.4a *Light dependence of modification in the Dl protein:* The induction of degradation of Dl is light dependent (Mattoo *et al* 1984; Salter *et al* 1992; Barbato *et al* 1992), but the degradation can proceed in total darkness after triggering event (Aro *et al* 1990). The degradation of Dl protein using PS II core preparations of Nanba and Satoh (1987) consisting of reaction center polypeptides CP47 and CP43 (Salter *et al* 1992) was studied. No proteolysis of Dl protein was observed unless photo-illuminated, suggesting that only the "light-triggered" Dl protein is subject to degradation (Salter *et al* 1992). PS II core particles photo-illuminated at low temperature were subject to degradation in dark at room temperature, suggesting that triggering of Dl is independent of degradation (Salter *et al* 1992).

The "light-triggered" Dl protein probably represents a modified form of Dl which is marked for degradation. The exact nature of light-triggered modification of Dl protein is not clear. However, the triggering event(s) leading to accumulation

of photodamaged D1 protein appear to be related to phosphorylation of D1 protein and/or conformational change(s) in the Dl protein induced by higher oxidizing species ($Z^+$ and $P680^+$) and oxygen-free radicals generated during photoinhibitory conditions. The loss of manganese (van-Wijk *et al* 1992) and the conversion of Dl protein into a low-mobility form designated as $Dl^*$ (Callahan *et al* 1990) are indicators of the conformational change(s) in the Dl protein. A conformational change in the Dl protein on the $Q_B$-binding site is involved in converting the Dl into a substrate for proteolysis (Andersson 1994).

(i) *Role of oxygen-free radicals:* The triggering event(s) appears to be co-related with modification in Dl protein brought about by oxygen-free radicals generated during photoinhibitory conditions in an oxygenic environment (Sopory *et al* 1990; Mishra *et al* 1994). The free radical scavengers propylgallate and uric acid were (Sopory *et al* 1990) and histidine and rutin (Mishra *et al* 1994) were shown to inhibit Dl degradation. Concomitant with degradation of Dl in core particles during exposure to high light in oxygenic environment, there was significant disappearance of CP43 and CP29 as well as photobleching of pigments (Mishra *et al* 1994). These results clearly establish that singlet oxygen and other oxygen-free radicals trigger a possible conformational change followed by proteolysis. However, data can not be taken as proof of autoproteolytic degradation of PS II reaction centre/core proteins.

(ii) *Phosphorylation induced modification of D1 protein:* The light dependent modification of Dl protein is its transformation to Dl* (Callahan *et al* 1990). Dl protein can be converted into Dl* *in vitro* in the non-photoinactivated thylakoids under conditions favouring protein phosphorylation (Aro *et al* 1993). In fact, Dl protein is one of the phosphoproteins observed *in vivo.* Thus, Andersson and co-workers (Aro *et al* 1993) concluded that the Dl* form seen during electrophoresis represents the phosphorylated form of the Dl protein. It is relevant to point out that the Dl* is present in the appressed membranes only (Callahan *et al* 1990) which are densely packed with PS II complex. Further, the ratio of Dl/Dl* is dependent on light intensity (see Aro *et al* 1993). However, conversion of Dl protein to Dl* is not a prerequisite for the degradation of Dl protein since no Dl* formation was observed in light in isolated thylakoids in which the PS II electron transport on donor or acceptor side had been knocked out (see Aro *et al* 1993). This is a erroneous conclusion since light dependent phosphorylation of PS II proteins is not likely to occur under these conditions since phosphorylation is dependent on the activation of the kinase controlled by the redox state of cyt $b_6$-f (see Dwivedi and Bhardwaj 1994).

PS II proteins Dl, D2. CP43, CP29, 9 kDa protein and the 27 and 25 kDa polypeptides of peripheral LHC II are known to be phosphorylated in light. It is interesting to note that except for the 9 kDa protein, these phosphoproteins are also subject to degradation in light (see also Andersson and Styring 1991). The degradation of phospho-LHC II in the unappressed regions of thylakoids has recently been shown (Andersson 1994; also personal communication).

It must be pointed out here that in many lower photosynthetic organisms, the light induced degradation of the Dl protein readily occurs without the phosphorylation of Dl protein (see Aro *et al* 1993). Thus, phosphorylation may not be prerequisite for Dl degradation in *vivo*.

3.4b *Dl degradation as an enzymatic process*: From the existing reports in literature, it is clear that light "triggered" or light activated Dl protein is a substrate for enzymatic degradation (and not simply photocleavage) since (i) the degradation could occur in complete darkness and at room temperature after previous exposure to high PFD under cold conditions (Aro *et al* 1990), (ii) the protein does not undergo a total cleavage and (iii) inhibitors of protease block the degradation of Dl protein. The degradation of Dl protein was inhibited by diisopropyl flourophosphate (DFP) (Virgin *et al* 1991) and it was shown that 43 kDa (CP43) binds one molecule of DFP (Salter *et al* 1992). These results led Andersson and coworkers (Salter *et al* 1992) to suggests that CP43 may act as a serine type of protease using "triggered" Dl as the substrate for proteolysis (Callahan *et al* 1990). It is pertinent to mention that CP43 contains numerous serines, histidines and aspartates, the typical relay system in serine proteases. However, the observed degradation of Dl protein in PS II reaction centre particles (Barbato *et al* 1991), which lack CP43 argues against involvement of CP43 in Dl protein degradation. It may be possible that one of the components of the reaction centre itself may act as a protease since Dl protein degradation has been observed using reaction centre particles. The involvement of components of reaction centre or core complex also appears to be doubtful since Dl protein and to a lesser extent D2 (Andersson 1994) as well as CP43 and CP29 (Mishra *et al* 1994) are themselves subject to degradation in high light. Since the degradation proceeds both from luminal (N-terminal) and stromal sides (C-terminal), more than one protease may possibly be involved.

Different patterns for Dl protein degradation have been reported under different experimental conditions (Barbato *et al* 1991; Aro *et al* 1993). The Dl protein is thought to be cleaved during light induced turnover *in vivo* at or close to the QEEE sequence (Greenberg *et al* 1987) or at residue 238, both located in the loop on the outer side between helices IV and V.

Light dependent proteolysis of Dl protein gives rise to primary degradation fragments 23 and 16 kDa, in addition to 14, 13 and 10 kDa fragments (Salter *et al* 1992). Using $^{32}$P-labelled Dl protein and sequence specific antisera, 23 and 16 kDa fragments were found to be degradation products originating from N- and C-termini of Dl protein (Salter *et al* 1992). The primary cleavage yielding N-terminal 23 kDa and a C-terminal 16 kDa is likely to occur at the exposed regions on the outer thylakoid surface between transmembrane helices IV and V of the Dl protein, as has been previously suggested (Mattoo *et al* 1989). The degradation products of Dl protein during donor side inactivation have been shown to be C-terminal 24 and 16 kDa fragments and an N-terminal 10 kDa fragment (Salter *et al* 1992; Barbato *et al* 1992).

The data of Friso *et al* (1993) suggest concurrent operation of both mechanisms leading to degradation of Dl protein. Further, since the Dl protein is cleaved both from C- and N-termini as well as from the outer and inner sides, more than one protease is expected to be involved. It remains to be established if CP43, acting as a serine type protease, also undergoes any modification/conformational change triggered by light, to be an active protease.

An aspect of photoinhibition which has not been touched in this review is the role of carotenoids in protection from photodamage. However, Dl turnover is known to occur even at low light intensities. The purpose of turnover of PS II proteins

cannot be photoprotection of PS II. It appears to be a consequence of the complicated PS II photochemistry in oxygenic environment and may result in down regulation of photosynthesis under intense light. Efforts should also be made to redesign Dl protein using site-directed mutagenesis, which will not be subject to photodamage.

## Acknowledgements

This work was supported in part by the Council of Scientific and Industrial Research, New Delhi, through an award of SRF to UD. The authors are also grateful to B Andersson for stimulating discussion and suggestions.

## References

Anderson J M and Osmond C B 1987 Shade sun responses: Compromises between acclimation and photoinhibition. in *Photoinhibition* (eds) D I Kyle, C B Osmond and C J Arntzen (Amsterdam; Elsevier) pp 1–38

Andersson B 1994 Proteolytic activities associated with photosystem II and its light harvesting apparatus; in *XVI Int. Congr. Biochem. Mol Biol.,* New Delhi, vol. 3. pp 37

Andersson B and Styring S 1991 Photosystem II; Molecular organization, function and acclimation; *Curr. Top. Bioenerg.* **16** 1-81

Aro E M, Hundal T, Carlbecg I and Andersson B 1990 *In vitro* studies on light induced inhibition of photosystem II and D-l protein degradation at low temperatures; *Biochim. Biophys. Acra* **1019** 269–275

Aro E M, Virgin I and Andersson B 1993 Photoinhibition of photosystem II motivation, protein damage and turnover; *Biochim. Biophys. Ada* **1143** 113-134

Asada K and Takahashi M 1987 Production and scavenging of active oxygen in photosynthesis; in *Topics in photosynthesis: Photoinhibition* (eds) D J Kyle. C B Osmond and C J Arntzen (Amsterdam; Elsevier) vol. **9,** 227–288

Barbato R, Shipton C A, Giacometti G M and Barber J 1991 New evidence suggests that the initial photoinduced cleavage of D-l protein may not occur near the PEST sequence; *FEBS Leu.* **290** 162-166

Barbato R A, Frizzo G, Friso F. Rigoni and Giacometti G M 1992 Photoinduced degradation of the Dl protein in isolated thylakoids and various photosystem II particles after donorside inactivations. Detection of a C-terminal 16 kDa fragment; *FFBS Leu.* **304** 136–140

Barber J, Chapman D J and Telfer A 1987 Characterization of a PS II reaction centre isolated from the chloroplasts of *Pisum sativum; FEBS Lett.* **220** 67-73

Barber J and Anderson B 1992 Too much of a good thing: Light can be bad for photosynthesis; *Trends Biochem. Sci.* **17** 61-66

Barry B A and Babcock 1987 Tyrosine radicals are involved in the proteolytic oxygen evolving system; *Proc. Natl. Acad. Sci. USA* **84** 7099-7103

Blobaugh D J and Cheniae G M 1990 Kinetics of photoinhibition in hydroxylamine-extracted photosystem II membranes identification of the sites of photodamage; *Biochemistry* **29** 5109–5118

Callaban F A. Ghirardi M L, Sopory S K, Mehta A M, Edelman M and Mattoo A K 1990 A novel metabolic form of the 32 kDa D-l protein in the grana localized reaction centre of photosystem II; *J. Biol. Chem.* **265** 15357–15360

Dostatni R. Meyer H E and Oettmeier W 1988 Mapping of two lyrosine residues involved in the quinone-($Q_B$) binding site of D-l reaction centre polypeptide of photosystem II; *FFBS I.ett* **239** 207–210

Dwivedi U and Bhardwaj R 1994 Cyrochrome *ba-f* complex; The carburettor of exciton distribution in oxygenic photosynthesis; *J. Biosci,* **19** 37–42

Eyal Y, Goloubinoff P and Edelman M 1987 The amino terminal region delimited by Met I and Met 37 is an integral part of the 32 kDa herbicide binding protein; *Plant Mol. Biol.* **8** 337–343

Formm H, Devic M, Fluhx R and Edelman M 1985 Control *of psbA* gene expression in nature *Spirodeta* chloroplasts. Light regulation of 32 kDa protein synthesis is independent of transcript level: *EMBO J.* **4** 291-295

Friso G, Giacometti G M, Barber J and Barbato R 1993 Evidence for concurrent donor and acceptor side photoinduced degradation of the Dl-protein in isolated reaction centres of Photosystem II; *Biochim. Biophys. Acta* **1144** 265-270

Funk C, Schroder W P, Green B R, Renger G and Andersson B 1994 The intrinsic 22 kDa protein is a chlorophyll-binding subunit of photosystem II; *FEBS Lett.* **342** 261-266

Greenberg B M, Gaba V, Mattoo A K and Edelman M 1987 Identification of a primary *in vivo* degradation product of the rapidly turning-over 32 kd protein of photosystem II; *EMBO J.* **6** 2865-2869

Gruissem W and Zurawski G 1985 Analysis of promoter regions for the spinach chloroplast *rbc L, atp B* and *pub A* genes; *EMBO J.* **4** 3375-3383

Horton P 1989 Interaction between electron transport and carbon assimilation: Regulation of light harvesting and photochemistry; *Photosynthesis Plant Biol. Ser.* **8** 393–406

Hundal T, Aro E-M, Carlberg I and Andersson B 1990 Restoration of light induced photosystem II inhibition without *de novo* protein synthesis; *FEBS Lett.* **267** 203-206

Inagaki N, Fujita S and Satoh K 1989 Solubilization and partial purification of a thylakoid enzyme of spinach involved in the processing of Dl protein; *FEBS Lett.* **246** 218-222

Jegerschold C, Virgin I and Styring S 1990 Light-dependent degradation of the Dl protein in photosystem II is accelerated after inhibition of the water splitting reaction; *Biochemistry* **29** 6179-6186

Jennings R C and Zucchelli G 1985 The influence of membrane stacking on light absorption by chloroplasts; *Photobiochem. Photobiophys.* **9** 215-221

Klein R R, Mason H S and Mullet J E 1988 Light regulated translation of chloroplast proteins. Transcripts of *psaA psaB, psbA* and *rbcL* are associated with polysmes in dark grown and illuminated barley seedlings; *J. Cell Biol.* **106** 289-301

Kuwabara T and Murata N 1982 Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II panicles of spinach chloroplasts; *Plant Cell Physiol.* **23** 533-539

Kuwabara T, Miyao M, Murata T and Murata N 1985 The function of 33-kDa protein in the photosynthetic oxygen evolution system studied by reconstitution experiments; *Biochim. Biophys. Acta* **806** 283-289

Kyle D J, Ohad I and Arntzen C J 1984 Membrane protein damage and repair: selective loss of quinone-protein function in chloroplast membranes; *Proc. Natl. Acad. Sci. USA* **81** 4070-4074

Kyle D J 1987 The biochemical basis for photoinhibition of photosystem II; in *Topics in photosynthesis* (eds) D J Kyle, C B Osmond and C J Arntzen (Amsterdam: Elsevier) vol. 9, pp 197-226

Link G 1982 Phytochrome control of plastid mRNA in mustard *(Sinapis alba* L); *Planta* **154** 81-86

Marder J B, Golonbinoff P and Edelman M 1984 Molecular architecture of the rapidly metabolized 32-kilodalton protein of photosystem II; *J. Biol. Chem.* **259** 3900-3908

Marder J B, Chapman D J, Telfer A, Nixon P J and Barber J 1987 Identification of *psbA* and *psbD* gene products, Dl and D2, as reaction centre proteins of photosystem 2; *Plant Mol. Biol.* **9** 325-333

Mattoo A K, Hoffman-Falk H, Marder J B and Edelman M 1984 Regulation of protein metabolism coupling of photosynthetic electron transport to *in vivo* degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes; *Proc. Natl. Acad. Sci. USA* **81** 1380-1384

Mattoo A K, Marder J B and Edelman M 1989 Dynamics of the Photosystem II reaction center; *Cell* **56** 41-246

Michel H, Hunt D F and Shabano Witz J 1988 Tandom mass spectroscopy reveals that three PS II proteins of spinach chloroplasts contain N-acetyl O-phosphothreonine at their N-termini; *J. Biol. Chem.* **263** 1123-1130

Mishra N P, Francke C, van Gorkam H J and Ghanotakis D F 1994 Destructive role of singlet oxygen during aerobic illumination of the photosystem II core complex; *Biochim. Biophys. Acta* **1186** 81-90

Mullet J E, Klein P G and Klein R R 1990 Chlorophyll regulates accumulated of the plastid-encoded chlorophyll-apoproteins CP43 and Dl by increasing apoprotein stability; *Proc. Natl. Acad. Sci. USA* **87** 4038-4042

Nanba O and Satoh K 1987 Isolation of a photosystem II reaction center consisting of D-l and D-2 polypeptide and cytochrome B559; *Proc. Natl. Acad. Sci. USA* **84** 109-112

Oettmeier W, Masson K and Johanningmeier U 1980 Photoaffinity labeling of the photosystem II herbicide binding protein; *FEBS Lett.* **118** 267-270

Ohad I, Kyle D J and Hirschberg J 1985 Light dependent degradation of $Q_B$-protein in isolated thylakoids; *EMBO J.* **4** 1655-1659

Powles S B 1984 Photoinhibition of Photosynthesis induced by visible light; *Annu. Rev. Plant Physiol.* **35** 15–44

Prasil O, Adir N and Ohad I 1992 The Photosystems: Structure, function and molecular biology; in *Topics in photosynthesis* (*ed.*) J Barber (Amsterdam: Elsevier), vol. 11, pp 220–250

Richter M, Ruhle W and Loild A 1990 Studies on the mechanism of photosystem II photoinhibition. I. A two step degradation of Dl protein; *Photosynth. Res.* **24** 229-235

Salter H A, Virgin I, Hagman A and Andersson B 1992 On the molecular mechanism of light induced Dl protein degradation in photosystem II core particles; *Biochemistry* **31** 3990-3998

Satoh K, Nakatani H Y, Stainback K E, Watson J and Amtzen C J 1983 Polypeptide composition of a photosystem II core complex. Presence of herbicide binding protein; *Biochim. Biophys. Acta* **724** 142–150

Schuster G, Shochat S, Adir N and Ohad I 1989 Inactivation of photosystem II and turnover of the D-l protein by light and heat stresses; *NATO ASI-Ser A; Life Sci. USA* **168** 499-510

Setlik I, Allakhverdiev S I, Nedbal L, Setlikova E and Klimov V V 1990 Three types of photosystem II photoinactivation. I. Damaging processes on the acceptor side; *Photosynth. Res.* **23** 39-48

Sopory S K, Greenberg B M, Mehta R A, Edelman M and Mattoo A K 1990 Free radical scavengers inhibit light-dependent degradation of the 32 kDa Photosystem II reaction center protein; Z. *Naturforsch.* **45c** 412-417

Styring S, Virgin I, Ehrenberg A and Andersson B 1990 Strong light photoinhibition of electron transport in Photosystem II Impairment of the function of the first quinone acceptor. $Q_A$; *Biochim. Biophys. Acta* **1015** 269-278

Takahashi M, Shirashi T and Asada K 1988 COOH-terminal residues of Dl and 44 kDa CPa-2 at spinach photosystem II core complex; *FEBS Lett.* **240** 6-8

Tang X S and Satoh K 1985 The oxygen evolving photosystem II core complex; *FEBS Lett.* **179** 60-64

Theg S M, Filar L J and Dilley R A 1986 Photoinactivation of chloroplasts already inhibited on the oxidizing side of photosystem II; *Biochim. Biophys. Acta* **849** 104-111

Trebst A 1987 The three-dimensional structure of the herbicide biniding niche on the reaction centre polypeptides of photosystem II; Z *Naturforsch.* **42c** 742-750

Trivedi P K, Nath P and Sane P V 1994 Characterization of *psbA* gene from chloroplasts of *Populus deltoides; J. Plant Biochem. Biotech.* **3** 97-102

van der Bolt F and Vermass W 1992 Photoinactivation of photosystem II as studied with site-directed D2 mutants of the cyanobacterium *Synichocystis* sp. PCC 6803; *Biochim. Biophys. Acta* **1098** 247-251

Vass I, Gatzen G and Holzwarth A R 1993 Picosecond time resolved fluorescence studies on photoinhibition and double reduction of $Q_A$ in photosystem II; *Biochim. Biophys. Acta* **1183** 388-396

van–Wijk K L, Andersson B and Styring S 1992 Spectroscopic characterization of photoinhibited photosystem II and kinetic resolution of the triggering of the Dl reaction centre protein for degradation; *Biochim. Biophys. Acta* **1100** 207-215

Virgin I, Styring S and Andersson B 1988 Photosystem II disorganization and manganese release after photoinhibition of isolated spinach thylakoid membranes; *FEBS Lett.* **233** 408-412

Virgin I, Ghanotakis D F and Andersson B 1990 Light induced Dl protein degradation in isolated photosystem II core complexes; *FEBS Lett.* **269** 45-48

Virgin I. Salter A H, Ghanotakis D F and Andersson B 1991 Light induced protein degradation is catalysed by a serine type protease; *FEBS Lett.* **287** 125-128

Virgin I, Salter A H, Hagman A, Vass 1, Styring S and Andersson B 1992 Molecular mechanisms behind light-induced inhibition of photosystem II electron transport and degradation of reaction centre polypeptides; *Biochim. Biophys. Acta.* **1101** 139-142

Zurawski G, Bonhert H-J, Whitfield P R and Bottomley W 1982 Nucleotide sequence of the gene for the 32000-M, thylakoid membrane protein from *Spinacia oleracea* and *Nicotiana debneyi* predicts a totally conserved primary translation product of $M_r$ 38950; *Proc. Natl. acad. sci. USA* **79** 7699-7703