Chapter 7

INTERACTION OF HIGH LIGHT TREATMENT AND TEMPERATURE STRESS IN ISOLATED PHOTOSYSTEM II PARTICLES FROM WHEAT

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7.1 INTRODUCTION

Prolonged illumination of isolated chloroplasts with strong visible light leads to photoinhibition of photosynthesis which is manifested as a decrease in the $O_2$ evolution activity (Powles, 1984, Critchley, 1988). Measurements of the electron transport rates and fluorescence yield showed that photoinhibition is primarily due to an inactivation of the photosystem II activity. There is evidence that photoinhibition eventually leads to photodegradation of the D1 polypeptide (Reisman and Ohad, 1986; Greenberg et al., 1989; Mattoo et al., 1984; Kyle et al., 1984) which form the protein matrix for binding of the functional redox groups of the reaction centre of PSII (Trebst, 1986; Michel and Deisenhofer, 1988).

The molecular mechanism of photoinhibition remains to be elucidated. Although the photodegradation of the D1 protein under strong illumination has been reported but the identity of the primary target within PSII and the mechanism of its degradation is not clearly understood. It has been proposed that the semiquinone anion radical formed during the reduction of $Q_B$ periodically reacts to cause D1 protein turnover presumably via an active oxygen intermediate (Greenberg et al., 1987; Kyle et al., 1984; Schuster et al.,
1988). We have investigated the role of active oxygen species and provide the implication of oxygen free radicals in the photoinhibition of PSII particles.

Simultaneous exposure to chilling temperatures (0-12°C) and high PFD promotes photoinhibition of photosynthesis in a large number of chilling sensitive and chilling tolerant plants (Powles et al., 1983; Ogren et al., 1984; Yakir et al., 1985; Bongi and Long, 1987; Hodgson et al., 1987; Wise and Naylor, 1987). The mechanism of low temperature induced photoinhibition is not properly understood. Several workers have reported that oxygen contributes significantly to low temperature induced photoinhibition. Effort was also made to compare the mechanism of photoinhibition induced by illumination of isolated PSII particles at 25 and 5°C.

7.2 RESULTS AND DISCUSSION

7.2.1 Electron transport activity

PSII particles isolated from laboratory grown wheat leaves were characterized by the measurement of electron transport activity and polypeptide analysis by SDS-PAGE (Fig. 7.1). Exposure of wheat PSII particles to 1200 W.m⁻² caused a decrease in electron transport activity. H₂O to DCIP electron transport activity was completely inhibited after light treatment of the PSII particles for 30 and 20 min. at
Molecular weight (kDa)

Fig. 7.1 Densitometric scan of SDS-PAGE of the photosystem II particles isolated from wheat leaves.
25 and 5°C respectively (Fig. 7.2). The results show that the inhibition of activity was faster at 5°C as compared to that at 25°C. The higher extent of damage during photoinhibition at 5°C may be attributed to the low temperature induced photoinhibition of PSII particles. Similar results have been reported for intact leaves (Somersalo and Krause, 1990), chloroplasts (Barenyi and Krause, 1985) and cyanobacteria (Bittersmann et al., 1988). The reason for exacerbation of photoinhibition damage at low temperature has been suggested to be due to inhibition of alternative means of dissipation of excessive excitation energy at low temperatures (Oquist et al., 1987). These results may also be correlated to the reports of higher rates of production of active oxygen species during light treatment at low temperatures (Schoner and Krause, 1990).

In order to study contribution of active oxygen species in imparting photoinhibition damage to PSII particles we photoinhibited the PSII particles in presence of catalase and SOD either independently or in combination with each other. Photoinhibition treatments were given at 25 and 5°C to examine whether the mechanism of photoinhibition was same at both the temperatures.

Catalase provided a protection to PSII particles against photoinhibition damage at both 25 and 5°C. The pro-
tection of PSII particles from photoinhibition damage by catalase indicates the involvement of H$_2$O$_2$ in the inhibition of electron transport activity. It is apparent from Fig. 7.2a and 7.2b that the extent of protection was more at 25°C. These results are similar to that obtained for wheat chloroplasts where catalase was found less effective in providing protection at 5°C (Chapter 5) which could be explained by the photolability of catalase at low temperature (Cheng et al., 1981; Volk and Feierabend, 1989). The photoinactivation of catalase might lead to decrease in the scavenging of H$_2$O$_2$ generated during photoinhibition. The persisting H$_2$O$_2$ might lead to greater damage to PSII particles at 5°C.

SOD, which is known to scavenge superoxide radicals, also protected the PSII particles from photoinhibition damage. The loss of activity on exposure of PSII particles to 1200 W.m$^{-2}$ for 20 min. at 25°C was 72 and 59% in the absence and presence of SOD respectively. The same treatment at 5°C resulted in complete loss of activity on photoinhibition in the absence of SOD whereas only 61% inhibition was observed when the light treatment was given in the presence of exogenously added SOD. It is clear that SOD prevented the high light induced loss of electron transport activity at both the temperatures, however, the protection was
Fig. 7.2 Electron transport activity on photoinhibition of isolated wheat PSII particles (BRY type) in absence (open circles) and presence of catalase (filled circles), SOD (open triangles) and catalase+SOD (filled triangles). PSII particles were photoinhibited with 1200 W.m⁻² at 25°C (a) and 5°C (b). Chlorophyll concentration during photoinhibition was 100 µg.ml⁻¹. Each point represent mean of three separate experiments. The H₂O to DCIP electron transport activity of unstressed PSII particles was 592±24 µmol/mg Chl/h.
comparatively more at 5°C. Relatively less damage to PSII electron transport chain in presence of SOD indicates the involvement of superoxide radicals during photoinhibition of PSII particles. The higher extent of protection at 5°C might be due to increased rate of O$_2^-$ generation during photoinhibition at low temperature. Exposure of PSII particles to high light intensity results in the accumulation of reduced quinones (Styring et al., 1990) which may transfer electrons to molecular oxygen resulting in the formation of superoxide radicals (Kyle, 1987). At low temperature, electron transport in PSII is saturated at lower light intensities. Thus photoinhibition at 5°C may result in further increase in oxygen activation which could have deleterious effects on the functions of PSII complex.

The protection of electron transport activity from photoinhibition was maximum when light treatment was given in the presence of both catalase and SOD. The higher extent of protection could be because of scavenging of both H$_2$O$_2$ and O$_2^-$ radicals. Photoinhibition of PSII particles for 30 min at 25°C completely inhibited the electron transport activity whereas in presence of catalase and SOD retained 78% of the activity. The effects of simultaneous presence of catalase and SOD were also similar at 5°C. However, the extent of protection was less at 5°C which could be due to increased rate of oxygen activation and photoinactivation of
catalase. Photoinactivation of catalase might prevent the scavenging of $H_2O_2$ which, in turn, may inhibit the electron transport activity at low temperature.

The mechanism by which these enzyme might reduce the photoinhibition damage is scavenging of active oxygen species. In addition to $H_2O_2$ and $O_2^-$, catalase together with SOD is also known to scavenge hydroxyl free radicals (Elstner, 1982). Hydroxyl free radicals may be generated from $O_2^-$ and $H_2O_2$ in light (Youngman et al., 1983; Elstner, 1987; Asada and Takahashi). This reaction is facilitated by the presence of chlorophylls and iron which are already present in the PSII complex (Ghanotakis and Yocum, 1990).

Our results, therefore, indicate that scavenging of $H_2O_2$, $O_2^-$ and $OH^-$ during light treatment reduced approximately three-fourth of the damage. Photoinhibition of PSII particles results in the degradation of D1 protein (Kyle et al., 1984; Ohad et al., 1985) which has been reported to be mediated by active oxygen species by several workers (Greenberg et al., 1990). Although we have not directly monitored the photodegradation of D1 protein, but on the basis of the loss of electron transport activity, it may be concluded that active oxygen species which are generated in PSII complex play an important role in the expression of photoinhibition damage. The existence of an oxygen independent mechanism of
photoinhibition is also possible since the scavenging of active oxygen species could not retain the activity to the initial level.

7.2.2 Chlorophyll fluorescence

Intrinsic chlorophyll fluorescence of isolated PSII particles were used as the second parameter to study the mechanism of photoinhibition at 25 and 5°C. There was a reduction of $F_v$ on photoinhibition of PSII particles at both the temperatures (Fig. 7.3). The reduction in $F_v$ was due to a decrease in $F_m$ and an increase in $F_o$ (data not shown). Physiologically, a reduction in $F_m$ indicates a structural alterations in PSII and $Q_B^-$ destabilization (Adir et al., 1990). The increase in $F_o$, which is a characteristic of photoinhibition damage which might occur due to a loss in the efficiency of excitation energy transfer from light harvesting complex to the PSII reaction center (Bjorkman, 1987). The loss of $F_v$ was more on photoinhibition at 5°C than that at 25°C. This observation is consistent with our results of greater photoinhibition at low temperature. The reduction in $F_v$ at 25 and 5°C led to proportional decrease in $F_v/F_m$ ratio of PSII particles. $F_v/F_m$ ratio is shown to be directly proportional to the photosynthetic efficiency of PSII complex (Bjorkman, 1987; Baker and Horton, 1987). Hence, our results suggest a reduction in PSII photochem-
Fig. 7.3 Change in variable chlorophyll fluorescence ($F_v$) on photoinhibition of isolated wheat PSI1 particles in absence (open circles) and presence of catalase (filled circles), SOD (open triangles) and catalase+SOD (filled triangles). The PSI1 particles were photoinhibited with 1200 W.m$^{-2}$ at 25°C (a) and 5°C (b). Chlorophyll concentration during photoinhibition was 100 µg.ml$^{-1}$. Each point represents the mean of three separate experiments.
Fig. 7.4 $F_v/F_m$ ratio of isolated wheat PSII particles on photoinhibition in absence (open circles) and presence of catalase (filled circles), SOD (open triangles) and catalase+SOD (filled triangles). The PSII particles were photoinhibited with 1200 W.m$^{-2}$ at 25°C (a) and 5°C (b). Chlorophyll concentration during photoinhibition was 100 µg.ml$^{-1}$. Each point represents the mean of three separate experiments.
istry on photoinhibition which could be due to loss of electron transport activity possibly because of photodegradation of the D1 protein.

The loss of chlorophyll fluorescence parameters $F_v$ and $F_v/F_m$ ratio was prevented by catalase and SOD present during photoinhibition. The protection provided by catalase was less at 5°C than at 25°C which could be due to its photoinactivation at low temperature. The effect of SOD was similar at both the temperature and it significantly inhibited the loss of $F_v$ and $F_v/F_m$ ratio (Fig. 7.3 and 7.4). The maximum protection was provided to PSII particles when both catalase and SOD were present during photoinhibition. Similar to the case of electron transport activity, the quenching of active oxygen species by catalase and SOD could not provide complete protection of the chlorophyll fluorescence parameters. These results again indicate the existence of an oxygen independent mechanism of photoinhibition of PSII complex at both the temperatures.

7.2.3 Lipid peroxidation

Lipids are considered as the structural components of the photosynthetic apparatus which play important role in the maintenance of optimal conformation of thylakoid proteins (Anderson, 1985; Murphy, 1986). One of the characteristic
features of thylakoid lipids is the high percentage of poly-
unsaturated fatty acyl residues (Murphy, 1986) which could
be sensitive to the active oxygen species generated during
photoinhibition. The peroxidative degradation of lipids
associated with Triton X-100 treated PSII enriched thylakoid
membrane fragments was followed in order to examine the
peroxidative action of active oxygen species.

Lipid peroxidation in PSII membranes increased linearly
with increase in the duration of light treatment at 25 and
5°C (Fig. 7.5). The extent of lipid peroxidation was higher
on photoinhibition at 5°C which indicates a greater extent
of oxygen activation during photoinhibition at low tempera-
ture. Catalase and SOD inhibited the peroxidation process
which might be due to scavenging of the toxic oxygen
species. Photoinactivation of catalase under high light
stress at low temperature failed to scavenge H₂O₂ which was
manifested as increase in lipid peroxidation during photo-
inhibition of PSII particles at 5°C in the presence of
catalase (Fig. 7.5b). Lipid peroxidation was the least when
photoinhibition was carried out in presence of both catalase
and SOD. However, the peroxidation was more at 5°C than that
at 25°C which indicates an increased production of active
oxygen species at low temperature. Moreover, the lack of
complete inhibition of lipid peroxidation in presence of
Fig. 7.5 Lipid peroxidation in wheat PSII particles on photoinhibition in absence (open circles) and presence of catalase (filled circles), SOD (open triangles) and catalase+SOD (filled triangles). The PSII particles were photoinhibited with 1200 W.m$^{-2}$ at 25°C (a) and 5°C (b). Chlorophyll concentration during photoinhibition was 100 μg.ml$^{-1}$. Each point represents the mean of three separate experiments.
catalase and SOD might indicate the generation of other toxic oxygen species (such as singlet oxygen) capable of inducing lipid peroxidation which could not be scavenged by these enzymes.