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Alteration in the acceptor side of photosystem II of chloroplast by high light

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Abstract. The effect of high light on the acceptor side of photosystem II of chloroplasts and core particles of spinach was studied. Both V_{max} and apparent K_m for DCIP were altered in photoinhibited photosystem II core particles. The double reciprocal plot analysis as a function of actinic light showed increased slope in chloroplasts photoinhibited in the presence of DCMU. Exposure of chloroplasts to high light in the presence of DCMU did not protect the chloroplast against high light induced decrease in F_m, level. Further the high light stress induced decrease in F_m level was not restored by the addition of DCMU. These results suggest that the high light stress induced damage to chloroplast involves alteration in the binding site for Q_B on the DI protein on the acceptor side of photosystem II.

Keywords. Acceptor side damage; photosystem II; high light stress; fluorescence.

1 Introduction

Inspite of essential requirement of light for the functional activity, high light inhibits chloroplast electron transport, resulting in a significant decrease in photosynthesis (Powles 1984). Photosystem II (PS II) is primary target of attack when plants are exposed to excessive light intensities (Powles 1984; see also Dwivedi and Bhardwaj 1995). The impairment of PS II electron transport under excessive light intensites than encountered during growth has been shown to result due to functional impairment of Q_A or Q_B on the acceptor side (the acceptor side mechanism) of PS II or due to accumulation of the highly oxidized species on the donor side of PS II (the donor side mechanism) (see Dwivedi and Bhardwaj 1995). The impairment/inhibition of PS II electron transport triggers the degradation of a number of PS II core proteins (see Dwivedi and Bhardwaj 1995).

In this communication we provide evidence to prove that high light stress alters the acceptor side of D1 protein.

2. Materials and methods

2.1 *Isolation of chloroplasts*

Type C chloroplasts (Hall 1972) were isolated from spinach leaves purchased from local market. Leaves were washed in distilled water and kept at 0 to 4°C and were used ithin 24 h. Deveined spinach leaves were cut into small pieces and homogenized in a Waring blendor at its maximum speed for 10 s in an isolation medium containing

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20 mM Tris-Cl buffer (pH 7·4), 10 mM $MnCl_2$, 5 mM NH_4 Cl, 5 mM $MgCl_2$, 400 mM sucrose and 1 mg/ml bovine serum albumin (Sigma, fraction V) and the homogenate was filtered through four layers of cheese cloth. The filtrate was centrifuged at 3000 *g* for 5 min. The supernatant was discarded and the pellet was suspended in the minimal volume of isolation medium.

2.2 *Estimation of chlorophylls*

The chorophyll (chl) content of chloroplasts and PS II core particles was determined according to Arnon (1949).

2.3 *Measurement PS II activity* (*DCIP-Hill reaction*)

The photoreduction of DCIP ($H_2O \rightarrow$ DCIP) was measured spectrophotometrically at 605 nm. Chloroplasts were suspended in a reaction mixture in a final volume of 3 ml containing 20 mM Tris-Cl buffer (pH 7.4), 5 mM $MgCl_2$, 5 mM $MnCl_2$, 5 mM NH_4 CI, 100 μM DCIP and chloroplasts equivalent to 20 μg chl/ml. The photoreduction of DCIP was measured after 30 s of illumination with saturating (I = 700 Wm^{-2}) white light. A slide projector (Kinderman, Germany) was used to obtain actinic light intensity. Neutral density glass filters were used to reduce the light intensity. The rate of DIP reduction was calculated using an extinction coefficient of 21 mM^{-1} cm^{-1}.

2.4 *Isolation of PS II core particles*

PS II core particles were isolated according to the procedure of Kuwabara and Murata (1982).

2.5 *Photoinhibitory treatments*

Photoinhibitory treatment (PIT) was carried out for different time period by exposing chloroplasts/PS II core particles suspended in the isolation medium at a concentration of 1400 μg chl/ml to high light (1700 Wm^{-2}). In order to characterize high light induced damage to the acceptor side of PS II, chloroplasts suspended in the isolation medium at chl concentration of 1400 μg/ml, were exposed to high light (1700 Wm^{-2}) in the presence and absence of DCMU (5 μM) for 6 min and then washed twice with the isolation medium followed by the measurement of PS II activity. PS II core particles exposed to high light for 8 min (t 1/2 = 8 min) were used to characterize the changesin the binding affinity of DCIP on the acceptor side of PS II.

3. Results

In order to understand the acceptor side induced inhibition of PS II electron transport by high light stress, chloroplasts were exposed to high light for increasing time in the presence and absence of Diuron (DCMU). In the presence of DCMU, the inhibition of PS II activity by high light stress was greater as compared to inhibition in its absence under aerobic condition (figure 1).

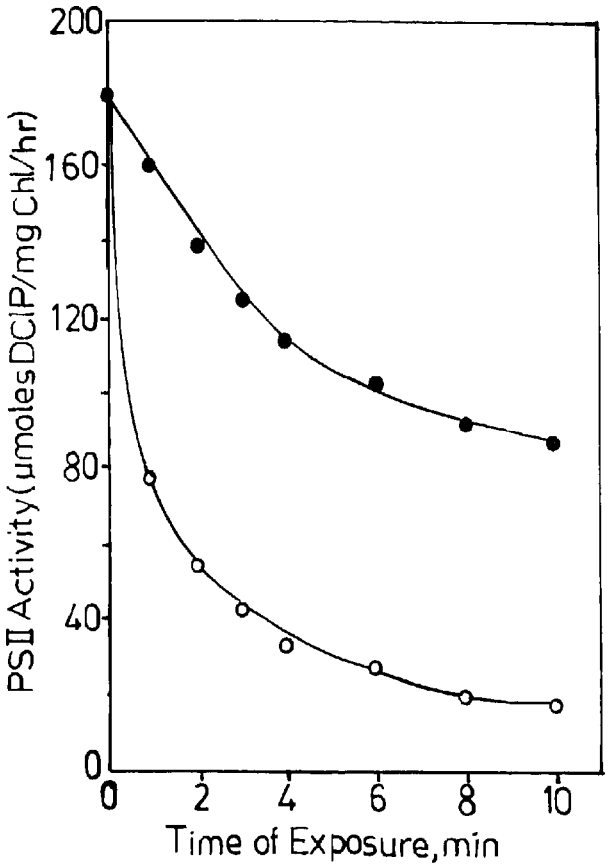

Figure 1. The effect of high light stress on PS II activity of chloroplasts exposed to high light at 1700 Wm^{-2} in the presence (5 μM) and absence of DCMU. Isolated chloroplasts were exposed to high light in the presence and absence of DCMU and then washed twice before measurement of PS II activity. (●), Minus DCMU. (O), Plus DCMU.

In order to understand the changes in the acceptor side of PS II due to high light stress, PS II activity of control chloroplasts and chloroplasts exposed to high light in the presence and absence of DCMU for 6 min was measured at limiting and saturating light intensities and the data was plotted in the form of double reciprocal plot (figure 2). The inhibition of PS II activity by high light was observed both at limiting and saturating light intensities. The double reciprocal plot analysis showed two parallel lines for control chloroplasts and chloroplasts photoinhibited in the absence of DCMU. The V_{max} decreased after photoinhibitory treatment (figure 2). However, there was no effect of the presence of DCMU during exposure to high light on the The V_{max} values were found to be same both in the presence and absence of DCMU during PIT (figure 2).

In order to characterize high light induced damage to the acceptor side in PS II core particles and the changes that affect the binding of DCIP on the acceptor side, PS II activity of control and treated PS II core particles was measured and the data was analysed by double reciprocal plot (figure 3). Both V_{max} and apparent K_m for DCIP were altered upon exposure of PS II core particles to high light. The apparent K_m for DCIP in control PS II core particles was calculated to be 66 ± 4 μM.

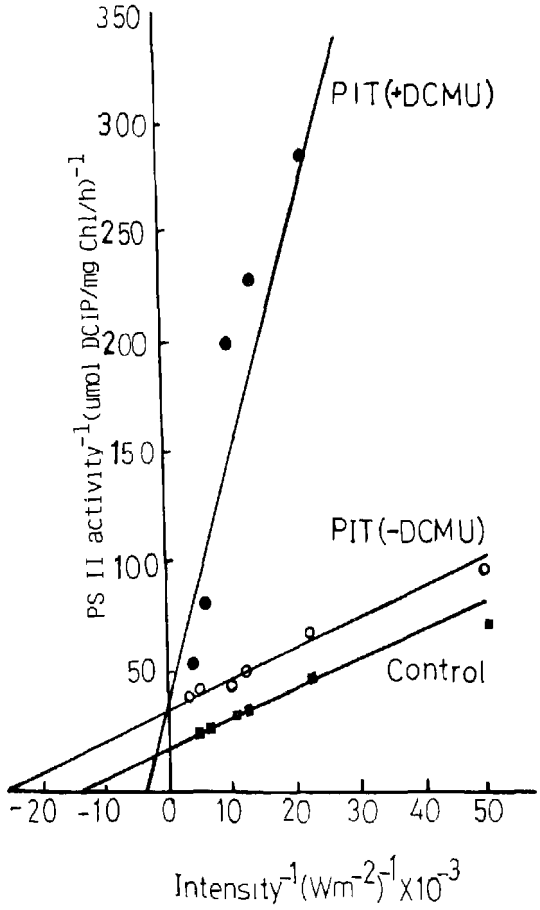

Figure 2. Double reciprocal plot analysis of the rate of PS II electron transport at limiting and saturating light intensity of control chloroplasts and chloroplasts exposed to high light at 1700 Wm $^{-2}$ for 6 min in the presence and absence of DCMU (5 μM). Note the change in slope in the presence of DCMU.

In order to characterize high light stress induced damage to PS II electron transport. steady state fluorescence yield of chl a *in vivo* was measured at room temperature (figure 4). Exposure of chloroplasts to high light lowered the F_m level (figure 4C). The typical PS decay was also found to be abolished when measurements were carried out in lower time scales (data not shown). Addition of DCMU to high light exposed chloroplasts did not restore the F_m level. There was no effect of the presence of DCMU during exposure to high light on F_m level (compare figure 4C with 4E).

4. Discussion

Two main mechanisms have been proposed to explain the high light induced inhibition of PS II electron transport (see Aro *et al* 1993; see also Dwivedi and Bhardwaj 1995). The first mechanism refered to as "acceptor side" mechanism, implies modification/impairment at the level of Q_A and/or Q_B (Kyle *et al* 1984; Setlik *et al* 1990). The

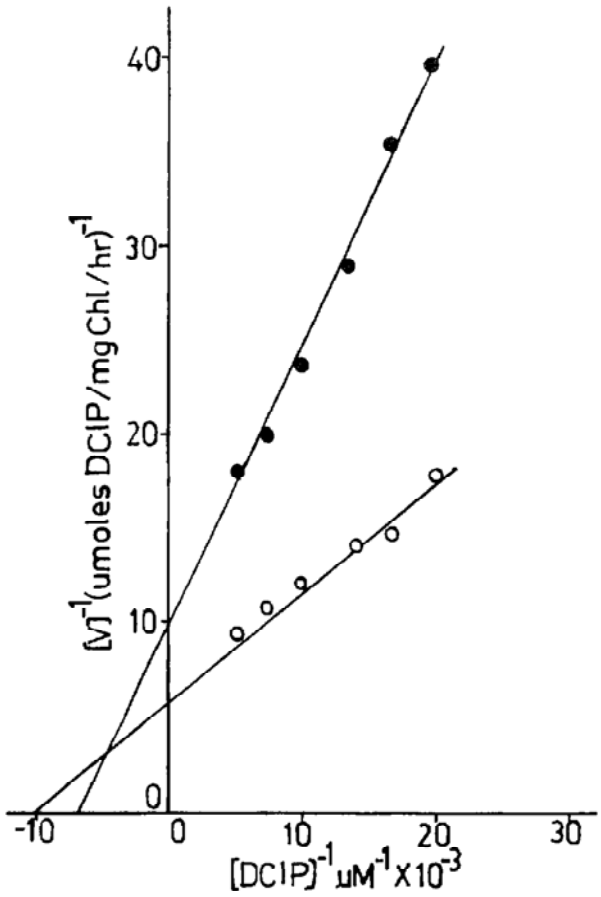

Figure 3. Double reciprocal plot analysis of rate of PS II electron transport in PS II core particles as a function of DCIP concentration. PS II activity of core particles was measured after exposing the particles to high light at 1700 Wm^{-2} for 8min. (●), Control. (O) Treated.

second mechanism refered to as "donor side" mechanism implies accumulation of highly oxidizing species on the donor side such as generation of Z^+ and/or $P680^+$ The decrease in fluorescence yield of chl *a in vivo* is related either with the inactivation of reaction centres or accumulation of oxidizing species since the fluorescence yield is controlled by the redox state of components on the acceptor side of PS II. Also Q_A/Q_B is known to leave D1 protein during high light stress affecting charge recombination. However, since F_0 level has not been found to increase (data not shown), it is suggested that high light primarily affects the reaction centre itself or the donor side of PS II. The decrease in the quantum yield of PS II by high light is well established (Dwivedi *et al* 1995). The binding characteristics of DCIP were altered in PS II core particles (figure 3). These results suggest modification of the acceptor side of PS II.

Diuron (DCMU) has been shown to block the recovery process which suggest that the recovery from photoinhibition involves re-establishment of electron transport between Q_A and Q_B (Mattoo *et al* 1981, 1984). The protective role of DCMU against photoinhibition has been reported by many workers (see Aro *et al* 1993) but there are evidence to prove that DCMU has no protective role (Mishra *et al* 1994). Thus, the role

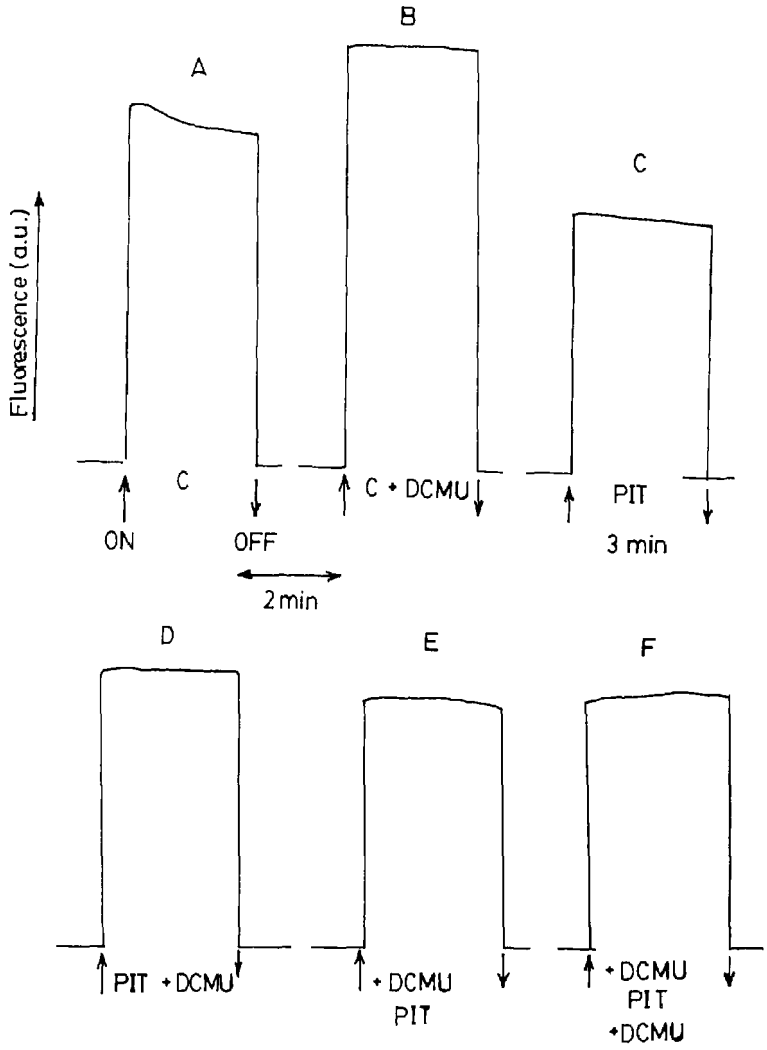

Figure 4. Steady state fluorescence yield of chl *a in vivo* of isolated control chloroplasts and chloroplasts exposed to high light for 3 min. The fluorescence was excited using a blue filter (cs 4-96) at 200 μEm^{-2} s^{-1}. Addition of DCMU to control chloroplasts (**A**) did not increase fluorescence amplitude but PS decay was abolished (**B**). In chloroplasts exposed to high light in the presence (**E**) and absence of DCMU (**C**), resulted in lowering of fluorescence yield as well as loss of PS decay (compare A vs C and A vs E). Addition of DCMU during fluorescence yield measurement did not restore the fluorescence level either in sample (**C**) or in sample (**E**).

of DCMU is controversial. DCMU was not found to have any protective role against photoinhibition (figures 1, 2) which is in agreement with the recent findings (Mishra *et al* 1994). The binding characteristics of DCMU were found to be altered when chloroplasts were exposed to high light (figure 2). These results suggest that the binding site of DCMU on D1 protein (Q_B) is modified as a result of high light stress.

DCMU did not afford any protection against high light induced damage as revealed by steady state fluorescence yield. There was no difference in the fluorescence yield when chloroplasts exposed to high light in the presence (figure 4E) or absence of

DCMU (figure 4C). DCMU is known to bind to Q_B and it is also known that Q_B leaves its site on D 1 during high light stress induced damage (see Aro *et al* 1993). Further, the high light stress induced damage is expected to be more in the presence of DCMU (due to block of electron transport and consequent increased photodamage). This further suggests that Q_B leaves its site.

This could be interpreted to mean that the D1 protein undergoes conformational modification(s) involving the Q_B site of D1 protein. Andersson (1994) has also suggested that high light induced modification of D1 protein involves the Q_B binding site of D1 protein turning the damaged protein into a substrat for proteolysis.

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