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Effect of Cations on Photosystem I Catalyzed Electron Transport Activity of Heat Stressed *Amaranthus* Chloroplasts

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The effect of elevated temperature stress (>25°C) on photosystem (PS) I catalyzed electron transport activity was studied in isolated *Amaranthus* chloroplasts. Incubation of chloroplasts at elevated temperature stimulated PS I activity supported by reduced-dichlorophenolindophenol, as electron donor. However, no such stimulation was noticed with reduced tetramethylpara-phenylenediamine or reduced-diaminodurene as electron donors. The stimulation of reduced dichlorophenolindophenol supported activity was observed at rate saturating light intensity but not at rate limiting low light intensity. The degree of stimulation was found to be dependent on cations but not on anions. Both mono- and di-valent cations like K^{+1}, Ca^{+2} and Mg^{+2} attenuated the activity. However, the extent of stimulation at saturating concentrations of cations, was greater with divalent than monovalent cations. The heat-induced alteration of thylakoid structure seems to require cations for screening of charges and stabilization of PS I activity.

Key Words: Chloroplasts, Heat stress, Photosystem I, Cation, *Amaranthus*

Introduction

The susceptibility of O_2 evolution associated with photosystem (PS) II in chloroplasts to temperature stress has long been recognized (Katoh & San Pietro 1967). Although it was suggested that the heat treatment induces inactivation of O_2 evolving complex, however, the failure in restoration of electron transport with substituted PS II electron donors (Yamashita & Butler 1968, Santarius 1975) indicated the involvement of structural changes in thylakoid membranes upon heat treatment. Recently it has been suggested that heat treatment causes destacking of thylakoid membranes (Gounaris et al. 1983), and the physical separation of light harvesting chlorophyll protein complexes (LHCP) from PS II core complex (Armond et al. 1980).

Such alteration of membrane structure by heat treatment induces stimulation of photo-electron transport rates catalyzed by PS I, (Emmett & Walker 1969, Armond et al. 1977, Sabat et al. 1986). The exact nature of this stimulation of PS I activity is not fully known. According to Ivanov et al. (1985) the heat treatment causes the migration of LHCP complex from grana to PS I enriched stroma lamellae thus increasing the absorptive cross section of PS I; and inducing enhancement in PS I activity. However, Thomas et al. (1986) have suggested that an increased flow of electrons to PS I from an intermediate close to plastoquinone is the cause for heat induced enhancement of PS I activity. We have recently also observed stimulation of reduced catechol supported PS I activity upon heat treatment (Sabat et al. 1986). In this paper, we present results on the effect of cations on PS I associated electron transport with reduced-dichlorophenolindophenol ($DCPIPH_2$) as electron donor in heat treated *Amaranthus* chloroplasts. Cations attenuate the $DCPIPH_2$ supported PS I catalyzed electron transport activity in heat treated chloroplasts.

Materials and Methods

Amaranthus viridis leaf chloroplasts were isolated following the procedure as described by Sabat et al. (1986). Chlorophyll (Chl) was measured according to Arnon (1949). Heat treatment of chloroplasts was done as described by Sabat et al. (1986). PS I catalyzed electron transport activity was determined polarographically as O_2 uptake. In order to study the effect of cations on PS I catalyzed electron transport activity both the unheated (control) and heat stressed chloroplasts were spun down (7000×g; 5 min) and

washed twice with 4 volumes of sucrose solution (100 mM sucrose with minimum of Tris to maintain *p*H 7.6). The resulting pellet was finally suspended in salt free sucrose medium (*p*H 7.6). Other details are mentioned in the legends to figures and tables.

Results and Discussion

Incubation of *Amaranthus* chloroplasts for 5 min. above 25°C showed a marked stimulation of $DCPIPH_2$ supported PSI electron transport rates, when measured at 25°C under light saturating condition (figure 1). The electron transport rate was found to increase with increasing incubation temperature up to 50°C (see figure 1) followed by a decline there after (data not shown). The degree of stimulation over control was linear between temperature range of 35–45°C (see inset figure 1). Basing on this observation, we have routinely used 50°C for heat treatment in most of our experiments. Studies with different PS I electron donors indicated that the heat induced enhancement was restricted only to $DCPIPH_2$ but not to reduced-tetramethyl para-phenylenediamine ($TMPDH_2$) or reduced-diaminodurene ($DADH_2$) as electron donors (table 1). Furthermore, the stimulation was found to occur at rate saturating light intensities but not at rate limiting intensities (figure 2). Heat-induced stimulation of PS I activity was also found to be sensitive to $HgCl_2$ treatment (table 2). In the heated chloroplasts, $HgCl_2$ arrested the electron transport activity to a slightly greater extent (10%) than in control. These observations suggest that heat treatment does alter the flow of electrons via plastocyanin (Kimimura & Katoh 1972). These observations are in good agreement with earlier studies (Thomas et al. 1986) suggesting that some membrane modification on the oxidizing side of PS I induces stimulation of electron donation by reduced-DCPIP. Since cations are known to affect the electron transport activities, we have analyzed the effect of cations on the heat treated chloroplast photosystem I activity (Barr et al. 1983, Packham & Barber 1984). Addition of both mono and divalent

Table 1 *Photosystem I mediated electron transport activity of control and heat stressed (HS)* Amaranthus *chloroplasts associated with DCPIPH$_2$ or TMPDH$_2$ or DADH$_2$ to methylviologen (MV) reaction*

	μmole O_2 consumed (mg Chl)$^{-1}$hr^{-1}		
	$DCPIPH_2$—MV	$TMPDH_2$—MV	$DADH_2$—MV
Control	430 ± 10	1220 ± 11	988 ± 07
HS	1174 ± 15	1103 ± 13	898 ± 09

Electron transport rate was monitored polarographically as O_2 consumption at 25°C under saturating light intensity (≃480 Wm^{-2}). The reaction mixture constituents were similar as described for figure 1. The concentration of $TMPDH_2$ or $DADH_2$ was 0.05mM respectively. The observation was made from three independent experiments and the data was analyzed statistically with ± SE

Table 2 *Effect of $HgCl_2$ on PS I mediated electron transport activity of control and heat stressed (HS)* Amaranthus *chloroplasts*

	μmole O_2 consumed (mg Chl)$^{-1}$ hr^{-1}					
	$DCPIPH_2$ → MV		$TMPDH_2$ → MV		$DADH_2$ → MV	
	$HgCl_2$ −	$HgCl_2$ +	$HgCl_2$ −	$HgCl_2$ +	$HgCl_2$ −	$HgCl_2$
Control	445 (100)	156 (35)	1249 (100)	99 (8)	1137 (100)	— (0)
HS	1190 (100)	274 (23)	1201 (100)	108 (9)	822 (100)	— (0)

Both control and heat-stressed chloroplasts were incubated with $HgCl_2$ (Chl to $HgCl_2$ mole ratio = 1) in a sealed tube for 1 h in dark at 4°C. Electron transport rate was determined polarographically in 3 ml of reaction mixture containing 100 mM sucrose, 10 mM NaCl, 50 mM HEPES-NaOH (*p*H 7.8), 3 mM sodium ascorbate, 2 mM $MgCl_2$, 1 mM sodium azide, 0.5 mM methylviologen, 0.005 mM DCMU and 20 μg chlorophyll equivalent chloroplasts. The concentration of DCPIP, TMPD and DAD were 0.1 mM 0.05 mM and 0.05 mM respectively. Light intensity was ≃ 480 Watts m^{-1}; + and — represent the presence and absence of $HgCl_2$. The tabulated value represents the mean of three independent observations. The value in parenthesis the % remaining activity of control (-$HgCl_2$) taken as 100%

Table 3 *Effect of the addition of some divalent and monovalent cations on the PSI mediated electron transport activity of control and heat stressed (HS)* Amaranthus *chloroplasts*

	μmole O_2 consumed (mg Chl)$^{-1}$hr^{-1}					
	$CaCl_2$		KNO_2		KNO_3	
	−	+	−	+	−	+
Control	300	450	325	360	315	335
HS	450	985	480	656	462	536

The basic reaction mixture was similar as described for figure 3 concentraion of $CaCl_2$ KNO_2 and KNO_3 was 8,40 and 40 mM respectively. The data represent mean of three independent observations. − and + denotes the absence and presence of the salt

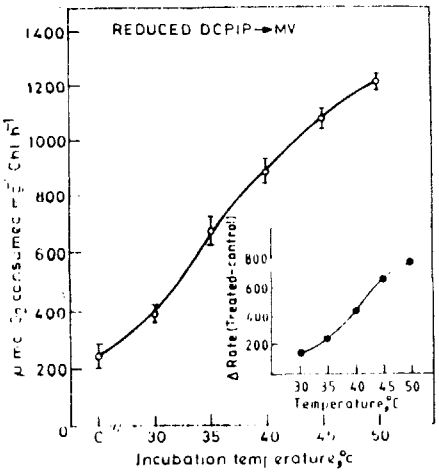


Figure 1 Temperature-dependent stimulation of PS I mediated electron transport rate of *Amaranthus* leaf chloroplsts during the transfer of electrons from $DCPIPH_2$ to methylviologen.

Isolated chloroplasts were incubated in the specified temperature for 5 min in dark. The electron transport rate was determined in terms of O_2 consumption in 3 ml of reaction mixture containing; 100 mM sucrose, 10 mM NaCl, 50 mM HEPES-NaOH (*p*H 7.8), 2 mM $MgCl_2$, 3 mM sodium ascorbate, 1 mM sodium azide 0.1 mM DCPIP, 0.5 mM MV, 0.005 mM DCMU and chloroplasts equivalent to 20 μg Chl, 'C' represents the unheated chloroplasts (control). **Inset:** Depicts the temperature-dependent change (Δ) in the rate of O_2 consumption with reference to control. Each value represents the mean of three independent observation from three different batches of isolated chloroplasts with standard error given as vertical bar. The assay was done at 25 ± 1°C under illumination (≃480 Watts m^{-2})

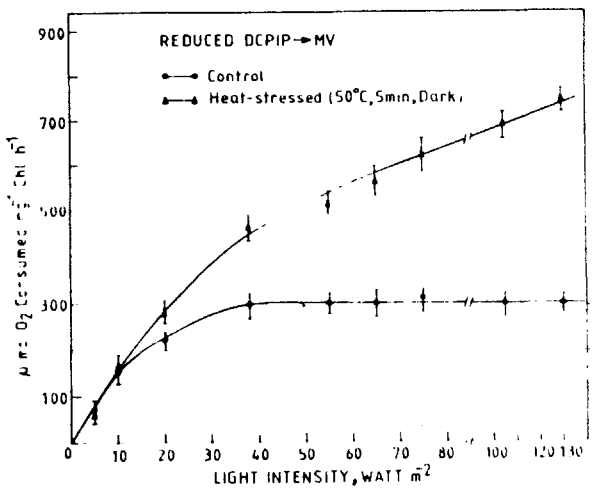
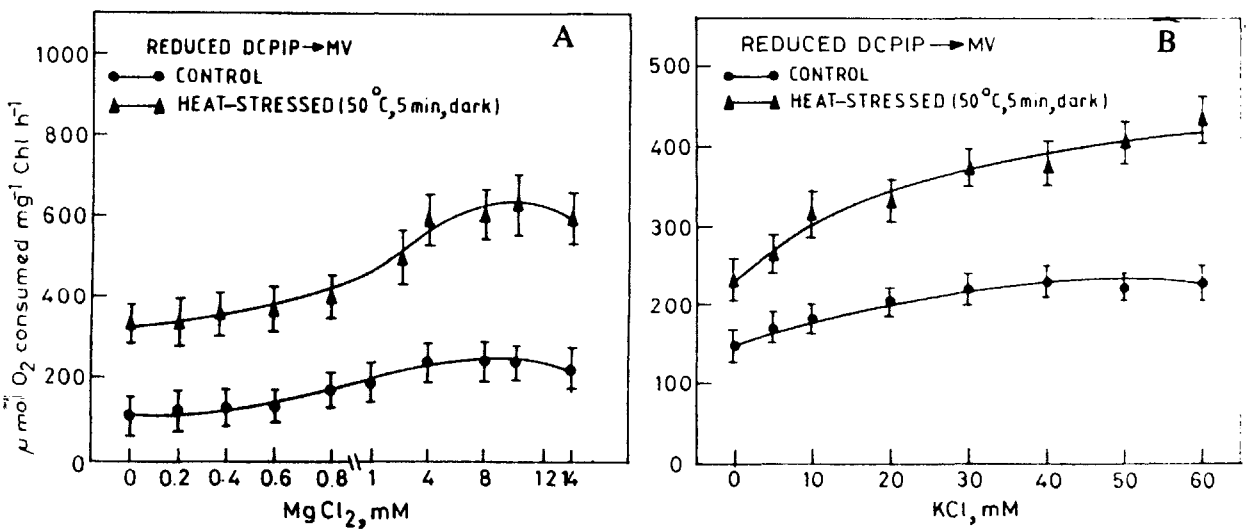


Figure 2 Effect of light intensities on the PS I catalyzed electron transport rate of control and HS *Amaranthus* chloroplasts. PS I activity was determined in terms of O_2 consumption in 3 ml reaction mixture containing similar reaction constituents as described for figure 1. Different light intensities were obtained by inserting calibrated filters between the light-source and the reaction vessel

Figure 3 (A–B) Effect of various concentration of $MgCl_2$ (**A**) and KCl (**B**) on PSI mediated electron transport activity of control and heat-stressed *Amaranthus* chloroplasts. Reaction mixture in a total volume of 3 ml contained; 100 mM sucrose (adjusted to pH 7.6 by addition of Tris), 0.1 mM DCPIP, 3 mM sodium ascorbate, 1 mM sodium azide, 0.005 mM DCMU, 0.5 mM MV and 20 μg chlorophyll equivalent chloroplasts. Light intensity and temperature were maintained at 480 watts m^{-2} and 25 ± 1°C respectively. Addition of ingradients to sucrose—Tris medium did not alter the set pH (pH 7.6). The concentration of $MgCl_2$ or KCl in the reaction mixture was as indicated in the figures



cations stimulated the PS I activity significantly in heat stressed chloroplasts as compared to control (figures 3A and 3B). Mg^{+2} or K^{+1} induced increase in PS I rate in control was only marginal. Table 3 shows that the stimulation in activity was dependent on cations and not on anions. Futhermore, the extent of stimulation, at saturating concentrations of the cations was greater with divalent than monovalent cations. Our results indicate that heat-induced alteration in thylakoid membrane needs cations for stabilization probably through charge screening. Tamura et al. (1980) have reported that cations regulate the accessibility of plastocyanin to the site of P_{700} reduction by screening the fixed negative charges of unstacked thylakoid menubrane. Under such circumstances, at alkaline assay *p*H the anionic form of DCPIP (Tripathy et al. 1984) could easily be accessible to its site of donation resulting in further attenuation in PS I activity.

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