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# Characterization of the heat-induced stimulation of Photosystem-I-mediated electron transport

OSAMA H. SAYED*, MICHAEL J. EARNSHAW and MICHAEL J. EMES

*Department of Cell & Structural Biology, Williamson Building, University of Manchester, Manchester M13 9PL, UK*

## SUMMARY

Uncoupled Photosystem I (PS I) activity driven by either reduced 2,6-dichlorophenolindophenol ($DCPIPH_2$) or N,N,N′,N′-tetramethyl-p-phenylene diamine ($TMPDH_2$) showed a stimulation following pre-treatment at 40–50°C, followed by inhibition at higher temperatures. The stimulation was more marked in thylakoids isolated from warm-grown plants than in those isolated from cool-grown plants. Approximately maximal rates of PS I activity were achieved at physiological temperatures (25–35°C) using highly lipophilic-reduced diaminodurene ($DADH_2$) as an electron donor with little stimulation occurring as a result of high temperature pre-treatment. Electron transport driven by all three electron donors was markedly inhibited by $KCN/HgCl_2$ following all pre-treatment temperatures, but relatively insensitive to ethyldimethylaminopropyl-carbodiimide (EDAC). It is suggested that the stimulation of PS I activity involves a phase change in the thylakoid membrane leading to an increase in permeability which allows enhanced access of $DCPIPH_2$ and $TMPDH_2$ to a common electron acceptor site located in the region of cytochrome f. The greater stimulation of electron transport in thylakoids isolated from warm-grown plants may be due to a more pronounced phase change occurring as a consequence of altered membrane composition as modified by growth temperature.

*Key-words:* Photosystem I, heat-induced stimulation, electron donor, *Triticum aestivum.*

## INTRODUCTION

The upper temperature limit for photosynthesis is, in most plants, marked by a sharp decline in photosynthetic efficiency. Photosynthetic inhibition under short-term heat stress (*c.* >40°C) reflects the susceptibility of the photosynthetic apparatus to heat damage rather than a decrease in stomatal conductance. Exposure to short-term heat stress has been shown to result in marked inhibition of oxygen evolution, carbon dioxide fixation, and photophosphorylation (Berry & Bjorkman 1980). Photosystem II (PS II)

*Correspondence author: Department of Botany, Faculty of Sciences, University of Qatar, Doha-Qatar, PO Box 2713.

activity was found to be particularly sensitive to heat stress (Santarius 1975; Sayed *et al.* 1989), whereas Photosystem I (PS I) activity appeared to be much more heat stable (Pearcy *et al.* 1977; Sayed *et al.* 1989). Photosystem I activity has widely been reported to be stimulated at temperatures above 35°C (Santarius 1975; Armond *et al.* 1978; Thomas *et al.* 1986), but the mechanisms involved are still unclear. Work described in this paper was designed to make use of various electron transport donors and inhibitors in an attempt to determine the nature of the heat-induced stimulation of PS-I-mediated electron transport. We have previously shown that the degree of stimulation of PS I activity following high-temperature pre-treatment is modulated by plant growth temperature (Sayed *et al.* 1989). Accordingly, all experiments were carried out using thylakoids isolated from cool- and warm-grown plants.

## MATERIALS AND METHODS

### *Plant material and growth regimes*

Spring wheat (*Triticum aestivum* L.) variety K65 (of Indian origin) was grown on vermiculite and watered with modified Hoagland's nutrient solution (Johnson *et al.* 1957) in growth cabinets under two growth regimes. The regimes applied were a cool regime (13/10°C) and a warm regime (30/25°C), with 12-h day/night cycles and an irradiance of 60 W $m^2$. Primary first leaves of the 16-day-old and 13-day-old plants grown under the cool and the warm regimes, respectively, were used for thylakoid isolation (Sayed *et al.* 1989).

### *Thylakoid isolation and pre-treatment*

Thylakoids were isolated using the method of Nolan & Smillie (1976) and were suspended in 1 ml of a medium containing 50 mM NaCl and 0·25% (w/v) bovine serum albumin. Chlorophyll content of thylakoid suspensions were determined after Arnon (1949). Freshly isolated thylakoids were separately treated with $KCN/HgCl_2$ and ethyldimethylaminopropyl-carbodiimide (EDAC), both treatments potentially inhibiting PS I activity (Trebst 1980). Cyanide-treated thylakoids were prepared by the method of Yocum (1980) and EDAC-treated thylakoids after McCarty (1974). Thylakoids were heat pre-treated at different temperatures for 3 min prior to measurement of electron transport at 25°C by incubating 50 µl aliquots of thylakoid suspension in 0·5 ml plastic tubes maintained in a temperature-controlled water bath.

### *Measurement of electron transport*

Photosystem-I-mediated electron transport uncoupled with ammonium chloride (1 mM) was estimated from the rate of oxygen uptake associated with the flow of electrons to methyl viologen (MV) from three different electron donors, namely reduced 2,6-dichlorophenolindophenol ($DCPIPH_2$), reduced N,N,N′,N′-tetra-methyl-p-phenylenediamine ($TMPDH_2$) or reduced diaminodurene ($DADH_2$) in an oxygen electrode (Hansatech, Norfolk, UK) at 25°C. The assay medium contained 1·0 ml of 50 mM Sorenson's phosphate buffer (pH 7·5), 50 mM NaCl, 0·8 µM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 2 mM sodium ascorbate, 50 µM MV, the electron donor (40 µM DCPIP, 0·1 mM TMPD or 0·5 mM DAD), and approximately 20 µg $ml^{-1}$ chlorophyll (Allen & Holmes 1986). Whole-chain electron transport

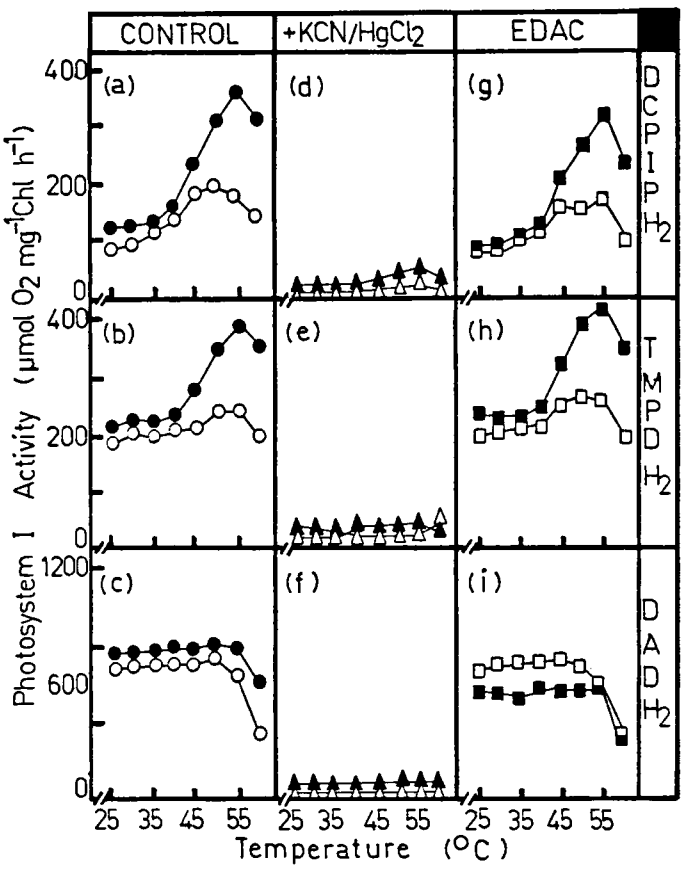


**Fig. 1.** The effects of $KCN/HgCl_2$ (50 μM/100 μM) and EDAC (1 mM) as a function of pre-treatment temperature on PS-I-mediated electron transport driven by $DCPIPH_2$, $TMPDH_2$, and $DADH_2$ in wheat thylakoids isolated from cool-grown plants (open symbols) and warm-grown plants (closed symbols). Note different scales for PS I activity.

($H_2O \rightarrow MV$) was determined as described previously (Sayed *et al.* 1989). All measurements were routinely repeated and data represent the mean of three measurements. Standard error bars were too small to be presented, and were omitted for clarity.

## RESULTS

The difference in rate of uncoupled PS-I-mediated electron transport ($DADH_2 > TMPDH_2 > DCPIPH_2$) over the physiological range of pre-treatment temperatures, i.e. 25–35°C (Fig. 1) is related to the respective decrease in lipophilicity of the electron donors (Sayed *et al.* 1989). Photosystem-I-mediated electron transport derived by $DCPIPH_2$ (Fig. 1a) and $TMPDH_2$ (Fig. 1b) exhibited a marked stimulation induced by pre-treatment temperatures in the range 40–50°C in thylakoids isolated from plants grown under the two growth regimes. As noted previously, the degree of stimulation was higher in thylakoids isolated from warm-grown plants than in those isolated from cool-grown plants (Sayed *et al.* 1989). However, the rate of PS-I-mediated electron

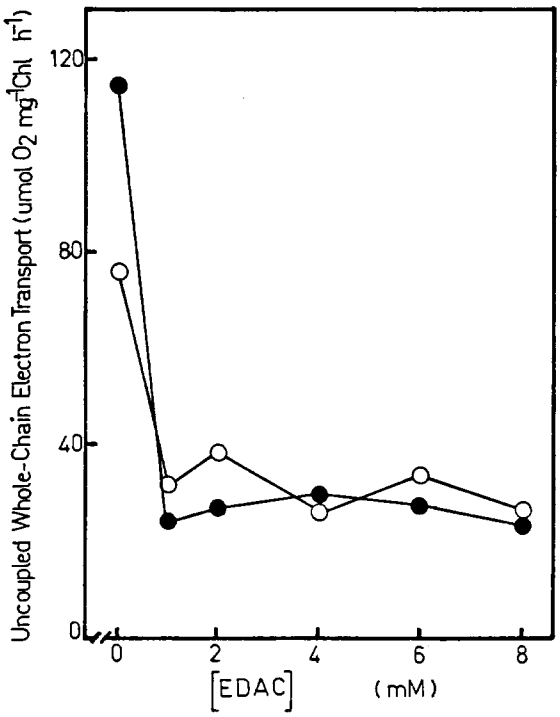


**Fig. 2.** The effect of EDAC concentration on whole-chain electron transport ($H_2O \rightarrow MV$) in wheat thylakoids isolated from cool-grown plants (open symbols) and warm-grown plants (closed symbols).

transport when driven by $DADH_2$ showed little stimulation in heat-stressed thylakoids isolated from either cool- or warm-grown plants (Fig. 1c). Photosystem I activity was inhibited in all cases at pre-treatment temperatures higher than *c.* 55°C.

The precise site of donation by the three electron donors used in this paper has previously been suggested to be in the cytochrome f (Cyt f)/plastoquinone (PQ) region (Larkum & Bonner 1972; Izawa 1980), plastocyanin, PC (Hauska 1977; Haehnel *et al.* 1981), or directly to P700 (Ke 1973). To gain further information on the donation site, the effects of electron transport inhibitors $KCN/HgCl_2$ and EDAC were examined. Treatment of thylakoids with $KCN/HgCl_2$ blocks electron transport by direct interaction with PC, and treatment with EDAC is thought to inhibit electron flow after PQ and before Cyt f and PC (Trebst 1980). The heat-induced stimulation of PS I activity was highly sensitive to the combined $KCN/HgCl_2$ treatment (Fig. 1d–f) as previously reported (Thomas *et al.* 1986). This treatment resulted in a dramatic reduction of *c.* 90% in PS I activity driven by the three electron donors within the physiological range of pretreatment temperatures and over the range of high pre-treatment temperatures (>40°C). In our hands, EDAC proved to be an effective inhibitor of whole-chain electron transport (Fig. 2). The rate of electron transport was inhibited by *c.* 65% in thylakoids isolated from cool-grown plants and *c.* 80% in those isolated from warm-grown plants. However, contrary to a previous report (Thomas *et al.* 1986), EDAC had little effect on heat-induced stimulation of PS-I-mediated electron transport driven by $DCPIPH_2$ (Fig. 1g) or by $TMPDH_2$ (Fig. 1h).

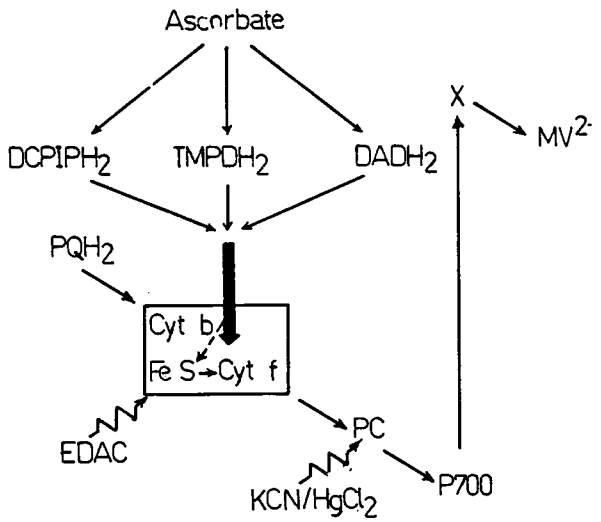


**Fig. 3.** Diagram illustrating the likely joint electron donation site of $DCPIPH_2$, $TMPDH_2$ and $DADH_2$ together with the points of action of $KCN/HgCl_2$ and EDAC in the photosynthetic electron transport chain. Based on Thomas *et al.* (1986), with the dashed line representing their proposed new acceptor site in heat-stressed thylakoids.

## DISCUSSION

The stimulation in heat-stressed thylakoids of PS I activity driven by $DCPIPH_2$ or $TMPDH_2$ as electron donors, followed by inhibition at high temperature, has previously been reported (Santarius 1975; Armond *et al.* 1978; Thomas *et al.* 1986; Sayed *et al.* 1989). This stimulation shows a threshold temperature similar to that of PS II inhibition by heat stress, but cannot be accounted for by thermal uncoupling alone as it takes place even in the presence of an uncoupler. The increase in PS-I-mediated electron transport is associated with a higher $V_{max}$ and a decrease in apparent $K_m$ for both $DCPIPH_2$ and $TMPDH_2$, implying increased accessibility of these donors to the electron transport chain (Sayed *et al.* 1989).

Thylakoids treated with $KCN/HgCl_2$ displayed an inhibition of about 90% of electron flow through PS I (Thomas *et al.* 1986) (Fig. 1). This inhibition of PS I activity lends support to the view that the primary site of donation by the three electron donors tested is located in the main electron transport chain, and that the suggested direct donation to P700 is a relatively unimportant process (Thomas *et al.* 1986). It is therefore suggested, following Thomas *et al.* (1986), that the electron-donation site at the Cyt $f/b_6$ complex is shared by the three electron donors tested as depicted in Fig. 3. Treatment with EDAC, on the other hand, had little effect on electron transport over the whole range of pre-treatment temperatures used (Fig. 1g–i). The reason for the difference between our results and those of Thomas *et al.* (1986) where EDAC substantially inhibited the high-temperature-induced stimulation of PS I activity is not apparent.

Reorganization of the thylakoid membrane as a result of heat stress clearly leads to an increase in accessibility of $DCPIPH_2$ and $TMPDH_2$ to the electron transport chain (Fig. 1). Thomas *et al.* (1986) favoured an explanation where membrane reorganization exposes new electron donation sites (Fig. 3, dashed line), rather than an increase in a thylakoid membrane permeability allowing enhanced access of the electron donor to

pre-existing acceptor sites. Their explanation rested partly on the finding that appreciable heat-induced stimulation of PS I activity occurred at a saturating concentration of $DCPIPH_2$ and was based on the assumption that Cyt f is fully accessible to the donor following high-temperature pre-treatment. However, we have shown that the rate of PS-I-mediated electron transport depends on donor lipophilicity (Fig. 1), where $DADH_2$ presumably fully saturates the acceptor site even at physiological temperatures, and we see no reason on the basis of our data to invoke the creation of additional sites as a result of high-temperature stress.

Incubation of thylakoids at high temperature results in the formation of membrane vesicles where the non-bilayer lipids form phase-separated aggregates of cylindrical inverted lipid micelles (Gounaris *et al.* 1984; Quinn & Williams 1985; Quinn 1988). This basic reorganization of thylakoid membrane structure is presumably responsible for increased penetration of $DCPIPH_2$ and $TMPDH_2$ to the acceptor site (Thomas *et al.* 1986). Moreover, the rate of electron donor penetration to Cyt f is clearly enhanced in thylakoids isolated from warm-grown plants (Fig. 1a,b). It seems reasonable to assume that a more pronounced phase separation occurs in these thylakoids, which is due to a change in membrane position brought about by growth temperature. Unfortunately, most studies of changes in membrane lipid composition as modulated by growth temperature have dealt with plasma membrane and/or tonoplast fractions (Clarkson *et al.* 1988; White *et al.* 1990) which have little relevance to the very different lipid composition of the thylakoid membrane. However, it appears that small amounts of high melting-point lipids are responsible for a high-temperature phase change in plant polar lipids with a transition temperature of *c.* 40°C (Raison & Wright 1983). Further, the increase in the low temperature phase transition that occurs in thylakoid polar lipids from warm-grown plants of *Nerium oleander* is associated with an increase in saturation of fatty acid species in phosphatidylglycerol and sulphoquinovosyldiacylglycerol (Orr & Raison 1987). Clearly, further work is needed along these lines in order to explain the enhanced penetration of PS I electron donors in thylakoids isolated from warm-grown plants.

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