S8-041

Effect of temperature and lipid composition on photoinhibition and recovery of higher plant Photosystem II

M Alfonso, R Collados and R Picorel

Estación Experimental de Aula Dei (CSIC), Avda Montañana 1005, 50059 Zaragoza, Spain.
picorel@eead.csic.es

Keywords: fatty acid, temperature, photoinhibition, Photosystem II

Introduction

Exposure of photosynthetic organisms to high light intensities decreases photosynthetic activity. This process includes the functional impairment of photosystem II (PSII) electron transport and the structural damage to the PSII reaction center (RC) D1 protein (Aro et al., 1993). Photosynthesis is also highly sensitive to thermal inhibition (Berry and Bjorkman, 1980). PSII is the most susceptible to heat among the various components of the thylakoid membrane (Berry and Bjorkman, 1980; Mamedov et al., 1993). The alleviation of thermal inhibition by low to moderate PAR (Schreiber and Berry, 1977) contrasted with the enhanced thermal injury at high light intensities (Al-Khathib and Paulsen, 1989) that increased the pressure on PSII. The injury inflicted by high temperature and high PAR was complementary whether both stresses were imposed simultaneously or sequentially, suggesting the possibility of a common locus for thermal inhibition and photoinhibition. Moreover, as noted by Powles (1982) intense heat combined with full sunlight typifies much of the earth's arable surface. Many studies concerning the interaction between low-temperature and photoinhibition have been published during the last years. These studies pointed to the role of membrane lipids in the tolerance to low temperature photoinhibition (reviewed by Nishida and Murata, 1996). In contrast, the interaction between high temperature inhibition and photoinhibition has been given little attention. We have studied the interaction between thermal inhibition and photoinhibition using the STR7 and WT soybean cell lines as a tool. The STR7 mutant presented herbicide resistance, increased sensitivity to light stress and an unusual tolerance to high temperatures probably due to a decrease in the unsaturation level of fatty acids from membrane lipids (Alfonso et al., 1996; Alfonso et al., 2001). The effect of both lipid composition and temperature on photoinhibition and recovery has been studied.

Materials and methods

Cell suspension culture conditions. Photosynthetic cell suspension cultures from the higher plant soybean (*Glycine max* var Corsoy) WT and STR7 mutant were grown in KN1 medium as previously described (Alfonso et al., 1996). Cells were cultured at 25 ºC under continuous light (75 $\mu E\ m^{-2}\ s^{-1}$) on a rotatory shaker at 130 rpm in a 5% CO_2 atmosphere.

In vivo high light and temperature experiments

For high light experiments, three week-old WT and STR7 cells were placed in a thermostated cuvette (AFORA, Spain) at the desired temperature. Cells were stirred during illumination with 1,000 $\mu E\ m^{-2}\ s^{-1}$ white light. Photoinhibition experiments were carried out at normal growth temperature (25 ºC) or 35 and 40 ºC. For recovery experiments, photoinhibited cells

were placed in the incubator shaker under normal growth conditions or placed in a similar incubator under the same growth conditions except that the temperature was increased to 35 or 40 ºC. Chloroplastic protein synthesis was inhibited by adding Chloramphenicol (Sigma) at a concentration of 300 μg mL^{-1}. Cells were incubated during 15 min in darkness before the high light or recovery treatment.

Chlorophyll fluorescence and photosynthetic activity measurements.

Chlorophyll fluorescence induction curves were recorded as previously described (Alfonso et al., 1996). Photosynthetic activity of thylakoid membranes isolated from WT and STR7 cells was measured at 25 ºC with a Clark-type oxygen electrode (Hansatech, UK) as previously described (Alfonso et al., 1996). DCBQ was used as an artificial electron acceptor.

Lipid analysis.

Total lipids were extracted from thylakoid with chloroform-methanol (2:1, v:v) as described by Bligh and Dyer (1959). Analysis of lipids and fatty acids was carried out as described previously (Alfonso et al., 2001).

Results and Discussion

Lipid and fatty acid composition from thylakoid membranes of WT and STR7.

Lipids isolated from the WT and STR7 mutant cells were analyzed to determine possible differences in fatty acid or glicerolipid composition. In general, there were no significant differences in the proportion of each glycerolipid component between WT and STR7 mutant (data not shown). However, significant differences were found between individual fatty acids of the respective glycerolipids from both cell lines (Table I).

Table I. Fatty acid composition (mol %) from total lipids isolated from WT and STR7 cells grown at 25 ºC.

	Fatty acid composition (mol %)							
	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$R_{sat/insat}$
WT	10.5	2.8	1.6	2.2	14.0	69.0	<0.05	0.137
STR7	20.1	<0.05	2.2	3.4	14.7	59.6	<0.05	0.287

When compared with WT, the STR7 mutant presented increased levels of 16:0 (around 20% of total fatty acids) while levels of 16:1 were nearly undetectable (< 0.05%) (Table I). We determined the saturated to unsaturated fatty acid ratio for all lipid classes. Table I shows that this ratio was always higher in the STR7 mutant. When these differences were analyzed for every lipid class, they were particularly high in chloroplastic MGDG, DGDG and PG lipids synthetized entirely in the chloroplast by the procariotic pathway (Alfonso et al., 2001).

Effect of temperature and protein synthesis inhibitors on in vivo photoinhibition in WT and STR7 mutant cells.

The *in vivo* photoinhibition of photosynthesis in WT and STR7 mutant cells was examined at 25, 35 and 40 ºC (Fig. 1) under a light intensity of 1,000 μE m^{-2} s^{-1}. At 25 ºC the STR7 mutant was more sensitive to photoinhibition than the WT (Fig. 1). STR7 mutant cells lost 50%

variable fluorescence after 90 min of photoinhibition while WT cells needed more than 250 min to reach a similar level of photoinhibition. When temperature was raised to 35 ºC the photoinhibition in both WT and STR7 mutant cells was already very similar (Fig. 1). At 40 ºC strong photoinhibition occurred in both WT and STR7 but the STR7 mutant was less susceptible to photoinhibition than WT at this temperature (Fig. 1). The STR7 reached a 50% photoinhibition after 120 min treatment while only 70 min were neccessary in WT.

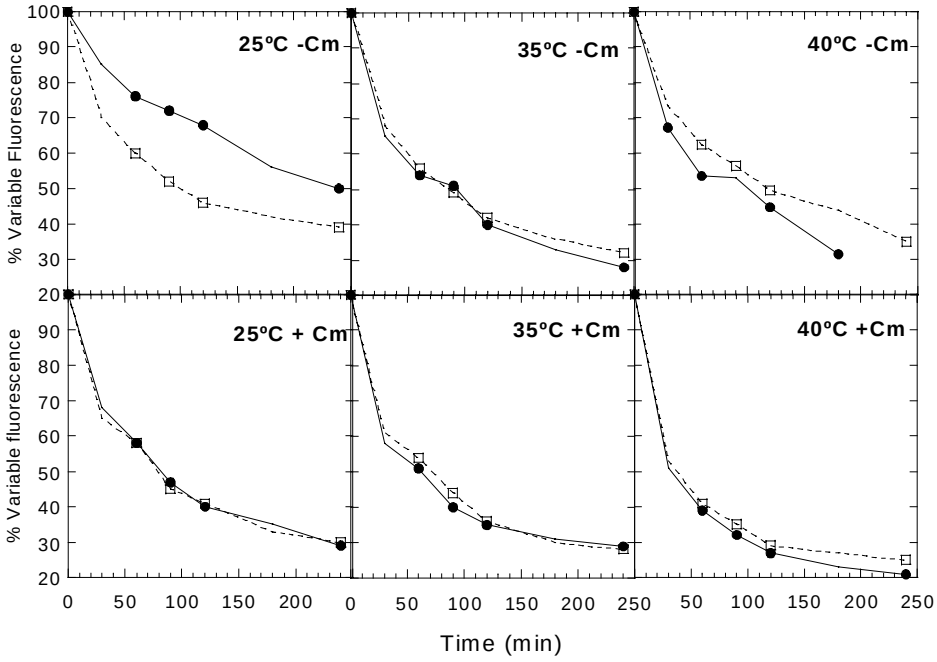

Figure1. Effects of high light and temperature on photoinhibition in WT and STR7 cells. Upper pannels (in the absence of Chloramfenicol), lower pannels (in the presence of Chloramphenicol. (•) WT, ( ) STR7 mutant. Data represent means of three independent experiments.

We examined the contribution of protein synthesis to photoinhibition performing similar experiments in the presence of Chloramphenicol, an inhibitor of protein synthesis in the chloroplast. WT and STR7 cells were incubated in the presence of Chloramphenicol (300 μg mL^{-1}) for 15 min at 25 ºC in darkness and then incubated in the light (1,000 μE m^{-2} s^{-1}) at 25, 35 and 40 ºC. Under these conditions, at all three temperatures studied there were no significant differences in the extent of photoinhibition between WT and STR7 cells (Fig.1). Data obtained at control temperature (25 ºC) indicated that photoinactivation, caused by the light-induced damage to the D1 protein was unaffected by the decrease in the level of unsaturated fatty-acids or by an altered Q_B site. Our data also indicated that the factor(s) responsible for the differences on the susceptibility to photoinhibition found between both cell lines was related to protein synthesis and assembly of the photosynthetic units.

Recovery of photosynthesis after photoinhibition at different temperatures

We decided to study the recovery from photoinhibition in WT and STR7 cells at 25, 35, and 40 ºC. WT and STR7 cells were photoinhibited (1,000 μE m^{-2} s^{-1}) till approximately 50%

total variable fluorescence was lost. Further photoinhibition time caused irreversible damage (data not shown). After photoinhibition, cells were incubated under normal light (70 $\mu E\, m^{-2}\, s^{-1}$) or in the dark under the same temperature for each photoinhibition experiment.

At 25 ºC, WT cells regained almost 100% of the original variable fluorescence (40% increase) after 120 min recovery (Fig. 2). At the same temperature, STR7 cells regained only 76% of the original activity (16% increase) after the same recovery time (Fig. 2). Almost no recovery was observed for both cell lines in the absence of light (Fig. 2) or in the presence of Chloramphenicol during illumination (data not shown). At 35 ºC, the recovery profile from photoinhibition was markedly different. WT cells showed only 22% increase of the variable fluorescence after a 120 min treatment (Fig. 2). Interestingly, the STR7 mutant recovered better at 35 ºC than at 25 ºC (Fig. 2). Indeed, STR7 cells regained up to 84% of the control values (34% increase of variable fluorescence) after a 120 min treatment. Again, no recovery was observed in the dark (Fig. 2) or in the presence of Chloramphenicol (data not shown) for both cell lines. These differences found at 35 ºC were even more pronounced at 40 ºC (18% increase in WT vs 30% increase in STR7, Fig. 2).

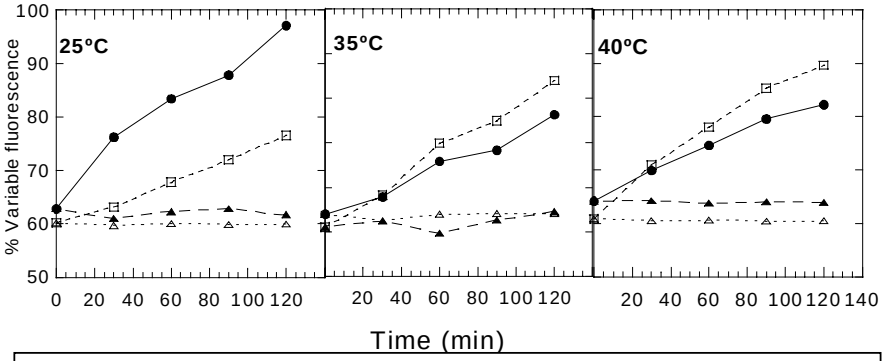

Figure 2. Recovery of PSII activity after photoinhibition in WT and STR7 cells at different represent means of three independent experiments.

The fact that recovery at 35 or 40 ºC was less affected than at 25 ºC indicated that changes in fatty acid unsaturation which may induce changes in membrane fluidity, found in STR7, would be responsible of this phenotype.

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