S8-007

A last-ditch defence against high-light stress: Photoinactivated photosystem II complexes protect functional neighbours in *Capsicum annuum* L. leaves

H.-Y. Lee[1,2,3], Y.-N. Hong[2] and W. S. Chow[1]
[1]*Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, ACT 2601, Australia. Email: chow@rsbs.anu.edu.au.*
[2]*School of Biological Sciences, Seoul National University, Seoul 151-742, South Korea.*
[3]*Present address: Isotope, Radiation Application Research Team, Korea Atomic Energy Research Institute, Taejon 305-353, South Korea.*

Keywords: photoinactivation, photoinhibition, photoprotection, photosystem II

Introduction

During normal photosynthesis, photoinactivation of photosystem II (PS II) and repair via *de novo* protein synthesis occur in parallel (see Chow 1994; Melis 1999). If repair cannot keep pace with photoinactivation, net loss of functional PS II ensures. A photoinactivated PS II may dissipate excitation energy efficiently as heat, preventing further damage to the complex itself (Krause 1988). Öquist et al. (1992) hypothesized that photoinactivated PS II complexes may protect neighbouring, connected PS II complexes from photoinactivation. However, attempts to test this hypothesis have not yielded positive results (eg. Tyystjärvi et al. 1994; Lee et al. 1999). The present study re-examines in detail the response of *Capsicum* leaf discs to light as a function of illumination time, at various irradiances and temperatures. The results support the above hypothesis, but only when prolonged illumination has photoinactivated a substantial fraction of the PS II complexes. (Full report, Lee et al. 2001.)

Materials and Methods

Capsicum annuum L. (cv. New Town No. 3) plants were grown in a potting mixture and watered daily with 'Aquasol' (Hortico Ltd, Australia) solution supplemented by iron chelate. The growth chamber was maintained at 24/21°C (12 h day/12 h night), the irradiance being 500 (high) or 100 (low) µmol photons m^{-2} s^{-1}.

Detached leaves were allowed to take up 3 mM lincomycin through the cut petiole in the dark for 3 h. The average concentration taken into the tissue was 2-3 mM. Leaf pieces were floated on distilled water or 1 mM lincomycin at a defined temperature. Illumination in normal air was provided at 460, 900 or 1,800 µmol photons m^{-2} s^{-1}. Typically, 15 leaf pieces were sampled at each time-point in three separate experiments to obtain a mean value.

Leaf pieces were sampled after an illumination treatment and dark-adapted for 30 min before measurement of F_o (corresponding to open PS II reaction centres) and F_m (closed reaction centres) using a Plant Efficiency Analyser (Hansatech, UK). Chlorophyll fluorescence yields after a light treatment (F_o and F_m) were all normalized to the F_o (set as 1.00) of leaf samples before illumination. The normalized parameter $1/F_o - 1/F_m$ was used as a linear indicator of the oxygen yield per single-turnover flash *Capsicum* (Lee et al. 1999).

The rate coefficients of photoinactivation (k_i) and repair (k_r) were determined from a kinetic model as described by Lee et al. (2001).

Results

Decline in the functional fraction of PS II (f) on illumination in the presence of lincomycin

During illumination of leaf pieces at 460, 900 or 1,800 µmol photons m^{-2} s^{-1} in the presence of lincomycin, *f* declined exponentially until it reached about 0.3 (semi-log plot in Fig. 1). Thereafter, the decline became slower, until a residual fraction of about 0.2 remained even though no repair was taking place. Various tests confirmed that the residual fraction did not escape the action of lincomycin (Lee et al. 2001). Therefore, the residual fraction must have been photoprotected in some way. We analysed the data (Lee et al. 2001) according to the hypothesis that photoinactivated PS II complexes photoprotect their functional neighbours. The analysis yielded k_i, an "intrinsic" rate coefficient of photoinactivation, for a suitable value of a parameter *a* that together with *f* helped to modify k_i.

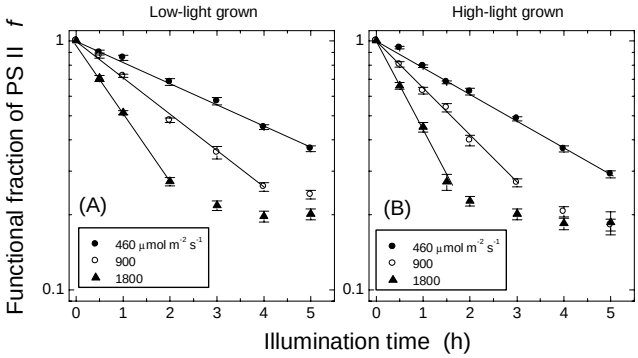

Fig. 1. Semi-log plots of the decline in the fraction of functional PS II during illumination in the presence of lincomycin at three irradiances. The data are fitted by a model (Lee et al. 2001) to obtain k_i.

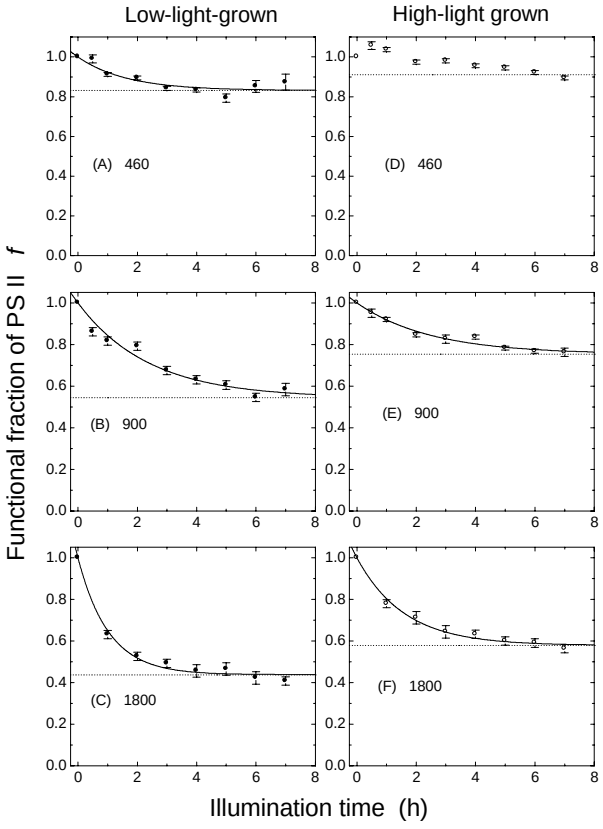

Fig. 2. Time course of decline in the fraction of functional PS II during illumination in the absence of lincomycin at 460, 900 or 1,800 µmol photons m^{-2} s^{-1}. Dotted lines indicate steady-state values f_S.

Decline in the functional fraction of PS II on illumination in the absence of lincomycin

During illumination of leaf pieces at 460, 900 or 1,800 µmol photons m^{-2} s^{-1} in the absence of lincomycin, f declined towards a steady value f_S in each case (Fig. 2). From the value of f_S (Fig. 2) and those of a and k_i as determined from Fig. 1, k_r could be estimated (Lee et al. 2001). The coefficients of repair and photoinactivation, obtained as a function of irradiance or temperature, are depicted in Fig. 3.

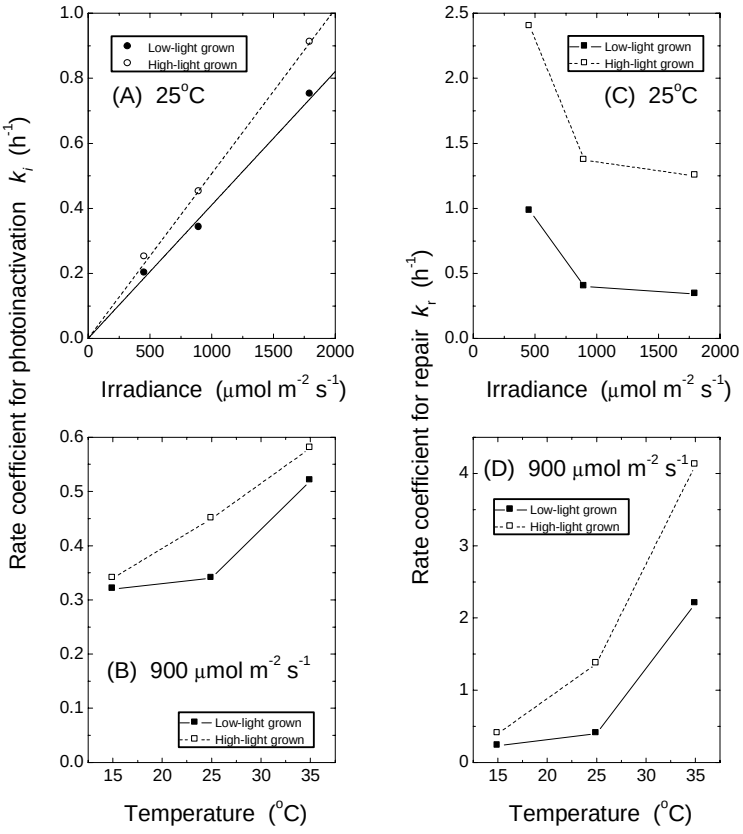

Fig. 3. Rate coefficients of photoinactivation (k_i) and of repair (k_r) as a function of treatment irradiance (A, C) or temperature (B, D). The coefficients were estimated by curve fitting according to Lee et al. (2001).

Discussion

Photoinactivated PS II complexes help to photoprotect connected functional neighbours

The data can be interpreted according to the hypothesis that photoinactivated PS II complexes help to photoprotect connected, functional neighbours (Öquist et al. 1992). Firstly, the retention of some functional PS II complexes during prolonged illumination even in the presence of lincomycin (Fig. 1) implies that the remaining functional PS II units derive photoprotection from mechanisms other than repair. Secondly, the decline in functional PS II complexes, during prolonged illumination in the presence of lincomycin, could be modelled by a varying operational rate coefficient, consisting of an "intrinsic" rate coefficient of photoinactivation k_i that was modified according to the increasing fraction of non-functional

PS II. Thus, as predicted by the hypothesis, the more abundant the non-functional PS II complexes, the greater is the degree of photoprotection. However, for the kinetic model to fit the data, it is assumed that any modification of the "intrinsic" rate coefficient of photoinactivation is minimal until a substantial fraction of PS II complexes has been photoinactivated.

Rate coefficient of photoinactivation of PS II in lincomycin-treated leaves (k_i)

Tyystjärvi and Aro (1996) showed that k_i is directly proportional to the irradiance. The linear increase in k_i with irradiance (Fig. 3A) agrees with their findings. Interestingly, for a given increase in treatment irradiance, the increase in k_i was about 20% less in low-light grown *Capsicum* than in high-light grown plants, perhaps due to better connectivity between functional PS II complexes and the dissipating, photoinactivated complexes, as has been hypothesized by Anderson and Aro (1994).

k_i was highest in leaf discs at 35°C (Fig. 3B): higher temperatures promoted photoinactivation of PS II, in agreement with findings with isolated thylakoids (Tyystjärvi et al. (1994).

Rate coefficient of repair (k_r)

As expected, k_r was highly dependent on temperature: it was very low at 15°C but increased several fold at 35°C, both in low-light and high-light grown plants (Fig. 3D). The accelerated repair at a higher temperature could be due to increased enzymic activity.

Another trend is a decrease in k_r with increase in irradiance from 460 μmol m^{-2} s^{-1} (Fig. 3C), implying slower rates of repair at higher irradiances. This is consistent with the results of Sundby et al. (1993). There is no clear explanation yet of why the rate coefficient for repair is lower at higher irradiances above growth irradiance, when repair is most needed.

Concluding remarks

The great abundance of photoinactivated PS II centres after prolonged illumination appeared to photoprotect residual functional centres. That such centres confer photoprotection of remaining functional PS II units may be extremely important for the survival of the leaf and, indeed, the plant. When favourable conditions return, it is expected that the functional complexes will help the non-functional counterparts to recover - a kind of mutual aid and cooperation.

Acknowledgments

This work was supported by the award of an Australian Government Scholarship (Korea) to H.-Y. Lee, and is part of the research supported by a grant from the Ministry of Education, South Korea (BSRI-1998-015-D00252).

References

Anderson, J. M. and Aro, E.-M.. (1994). *Photosynthesis Research* **41**: 321-326.
Chow, W. S. (1994). *Advances in Molecular and Cell Biology* **10**: 151-196.
Krause, G. H. (1988). *Physiologia Plantarum* **74**: 566-574.
Lee, H.-Y., Chow, W. S. and Hong, Y.-N. (1999). *Physiologia Plantarum* **105**: 377-384.
Lee, H.-Y., Hong, Y.-N. and Chow, W. S. (2001). *Planta* **212**: 332-342.
Melis, A. (1999). *Trends in Plant Science* **4**: 130-135.
Öquist, G., Chow, W.S. and Anderson, J.M. (1992). *Planta* **186**: 450-460.

Sundby, C., McCaffery, S. and Anderson, J. M. (1993). *Journal of Biological Chemistry* **268**: 25476-25482.

Tyystjärvi, E. and Aro, E.-M. (1996). *Proceedings of the National Academy Science USA* **93**: 2213-2218.

Tyystjärvi, E., Kettunen, R. and Aro, E.-M. (1994). *Biochimica et Biophysica Acta* **1186**: 177-185.