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BRIEF COMMUNICATION

Dissipation of excitation energy during development of photosystem 2 photochemistry in *Helianthus annuus*

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Abstract

Etiolated sunflower cotyledons developed in complete darkness and lacking photosystem (PS) 2 were exposed to continuous 200 μmol(photon) m^{-2} s^{-1} "white light" for 1, 3, 6, 12, and 18 h prior to evaluations of excitation-energy dissipation using modulated chlorophyll *a* fluorescence. Photochemical potential of PS2, measured as the dark-adapted quantum efficiency of PS2 ($F_{V(M)}/F_M$), and thermal dissipation from the antenna pigment-protein complex, measured as the Stern-Volmer non-photochemical quenching coefficient (NPQ), increased to 12 h of irradiation. Following 12 h of irradiation, thermal dissipation from the antennae pigment-protein complex decreased while the efficiency of excitation capture by PS2 centers (F'_V/F'_M) and light-adapted quantum efficiency of PS2 (Φ_{PS2}) continued to increase to 18 h of irradiation. The fraction of the oxidized state of Q_A, measured by the photochemical quenching coefficient (q_P), remained near optimal and was not changed significantly by irradiation time. Hence during the development of maximum photochemical potential of PS2 in sunflower etioplasts, which initially lacked PS2, enhanced thermal dissipation helps limit excitation energy reaching PS2 centers. Changes of the magnitude of thermal dissipation help maintain an optimum fraction of the oxidized state of Q_A during the development of PS2 photochemistry.

Additional key words: chlorophyll *a* fluorescence; etioplast; non-photochemical dissipation; sunflower.

During germination of angiosperm seeds in darkness, proplastids develop into pale-yellow etioplasts that lack the components necessary for photosynthesis. Conversion of etioplasts to chloroplasts requires irradiation-induced transformation of prolamellar bodies into thylakoids, conversion of protochlorophyllide to chlorophyll (Chl), and synthesis of the four major protein complexes of thylakoid membranes: photosystem (PS) 2, PS1, cytochrome-b_6f complex, and ATP synthase (Mullet 1988).

The most prominent mechanism of non-photochemical dissipation of excitation energy is thermal dissipation by the antenna pigment-protein complex of PS2 associated with deepoxidation of violaxanthin to zeaxanthin (for review see Gilmore and Govindjee 1999). The development of thermal dissipation in intermittent-light grown plants containing fully functional PS2 centers during continuous irradiation-induced synthesis of antennae pigment-protein complexes is well documented (Jahns and Krause 1994, Johnson and Krieger 1994, Chow *et al.* 2000). Under natural conditions, development of the antenna pigment-protein complex of PS2 coincides with the development of electron transport within PS2 as etiolated seedlings emerge from the soil and are exposed to continuous irradiation (Mysliwa-Kurdziel *et al.* 1997, Lebkuecher *et al.* 1999). This study characterizes changes of excitation-energy dissipation in relation to the development of PS2 photochemistry during continuous irradiation-induced development of chloroplasts from etioplasts that initially lack PS2.

Etiolated cotyledons from *Helianthus annuus* L. seedlings germinated in darkness for 6 d were exposed to 200 μmol(photon) m^{-2} s^{-1} "white light" provided by fluo-

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Abbreviations: Chl – chlorophyll; F_M – dark-adapted maximum fluorescence; F'_M – light-adapted maximum fluorescence; $F_{V(M)}$ – dark-adapted maximum variable fluorescence; F'_V – light-adapted variable fluorescence; NPQ – Stern-Volmer non-photochemical quenching coefficient; PS – photosystem; Q – quinone; q_N – non-photochemical quenching coefficient; q_P – photochemical quenching coefficient; Φ_{PS2} – light-adapted quantum efficiency of PS2.
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rescent, cool-white lamps at 25 °C for time periods of 1, 3, 6, 12, and 18 h. A modulated Chl *a* fluorescence system (*FMS 2*; *Hansatech*, Norfolk, UK) was used to evaluate fluorescence characteristics following the methods of Genty *et al.* (1989). Preliminary experiments employed additional irradiations of 9 and 24 h. Fluorescence characteristics of cotyledons exposed to 9 and 24 h of irradiation were not different from the 12-h and 18-h irradiations, respectively. Therefore, only the 1, 3, 6, 12, and 18 h treatments were chosen to eliminate unnecessary treatments which would reduce the chance of detecting statistically significant differences. Cotyledons were dark-adapted for 10 min. Dark-adapted maximum fluorescence (F_M) was determined by a 2-s pulse of 2 000 μmol(photon) m^{-2} s^{-1} "white light". Steady-state fluorescence was determined following 3 min of 200 μmol(photon) m^{-2} s^{-1} "white light" followed by a 2-s pulse of 2 000 μmol(photon) m^{-2} s^{-1} "white light" to measure maximum light-adapted fluorescence (F'_M). Light-adapted origin fluorescence was determined following the second 2-s pulse of 2 000 μmol(photon) m^{-2} s^{-1} "white light" by exposure to 5 s of far-red radiation (735-nm peak). The fluorescence parameters were calculated automatically by the *FMS 2*-fluorescence system and are identical to the calculations described in Deltoro *et al.* (1999). All treatment means were based on four replicates and were compared with each other by Tukey-Kramer Honestly Significant Difference Tests (Sokal and Rohlf 1995).

Dark-adapted variable fluorescence yield ($F_{V(M)}$), photochemical potential of PS2 (dark-adapted quantum efficiency of PS2; $F_{V(M)}/F_M$), and thermal dissipation of excitation energy by the antennae pigment-protein complex of PS2, measured as the Stern-Volmer non-photochemical quenching coefficient (NPQ), increased to 12 h of irradiation (Table 1). Following the cessation of increases of $F_{V(M)}$ and $F_{V(M)}/F_M$ at 12 h of irradiation, the NPQ decreased significantly. These results indicate that enhanced thermal dissipation by the antennae pigment-protein complex helps limit the number of excitons reaching PS2 centers during development of the maximum photochemical potential of PS2.

The characteristics of Chl *a* fluorescence of cotyledons exposed to 18 h of irradiation did not differ significantly from cotyledons of seedlings grown for 6 d under constant irradiation (values not shown). The light-adapted quantum efficiency of PS2 (Φ_{PS2}) is depressed by thermal dissipation and parallels the rates of non-cyclic electron transport and CO_2 assimilation (Sundberg *et al.* 1997). The continued increase of the efficiency of excitation-energy capture by PS2 centers (F'_V/F'_M) and Φ_{PS2} to maximum values during the 12-h to 18-h irradiation, although there were no significant changes of $F_{V(M)}$ and $F_{V(M)}/F_M$, reflects the delayed development of maximum photosynthetic capacity relative to maximum photochemical potential of PS2. This conclusion is consistent with several studies demonstrating that development of electron transport within PS2 precedes development of whole-chain electron transport and CO_2-assimilation capacity (Ohashi *et al.* 1989, Lebkuecher 1997).

The fraction of the oxidized state of Q_A, measured as the photochemical quenching coefficient (q_P; Ögren 1991), did not change significantly with irradiation time and varied within the range considered optimal in mature chloroplasts (Srivastava *et al.* 1995). Of course, numerous physiological changes occur during the development of photochemistry which affect dissipation of excitation energy, CO_2 assimilation, and thus Φ_{PS2} which are not addressed by this study. Several mechanisms help prevent sustained reduction of PS2 during development of photochemistry including PS2 state transitions (Webber and Baker 1996) and cyclic electron transport (Franck *et al.* 1995). The fluctuations of NPQ during the development of maximum values of Φ_{PS2} at 18 h of irradiation indicate that changes of thermal dissipation from the antenna pigment-protein complex help regulate the number of excitons that reach PS2 centers. In this way they help maintain an optimum fraction of the oxidized state of Q_A during the development of PS2 photochemistry. This conclusion is consistent with several studies which indicate that thermal dissipation of excess excitation energy from the antenna pigment-protein complex helps prevent complete reduction of Q_A in mature leaves during environmental conditions which limit photochemical dissipation of excitation energy (Demmig-Adams and Adams 1992, Eickmeier *et al.* 1992).

The low NPQ value relative to the non-photochemical quenching coefficient (q_N) value at 1 h of irradiation suggests that thermal dissipation at 1 h of irradiation originates largely from PS2 centers. Unlike the q_N, the NPQ parallels the magnitude of thermal dissipation from the antennae pigment-protein complex (Demmig-Adams *et al.* 1996) and is unaffected by non-photochemical dissipation by PS2 centers (Gilmore *et al.* 1995). Non-photochemical dissipation by PS2 centers is not fully understood (Gilmore and Govindjee 1999), however, it apparently results from recombination reactions between pheophytin$^-$ or Q_A^- and P_{680}^+ during limited water-splitting capacity (Schreiber *et al.* 1994, Laisk *et al.* 1997, Šiffel *et al.* 2000). Water-splitting activity and electron transport between Q_A^- and Q_B are very limited at 1 h of continuous irradiation of sunflower etioplasts, increase gradually to 6 h, and then dramatically to maximum values at 12 h of irradiation (Lebkuecher *et al.* 1999). These earlier results are consistent with the indication by the high q_N relative to NPQ value at 1 h of irradiation that non-photochemical dissipation results largely from PS2 centers during the earliest stages of the development of PS2 photochemistry.

Our results are consistent with those of Johnson and Krieger (1994). They demonstrated that non-photochemical quenching by PS2 centers is enhanced by Ca^{+2}-depleted PS2. Upon continuous irradiation the antennae pigment-protein complex is developed in intermittent-light

Table 1. Modulated chlorophyll *a* fluorescence characteristics of cotyledons from *Helianthus annuus* seedlings germinated in continuous darkness followed by 1, 3, 6, 12, and 18 h of irradiation [200 μmol(photon) m^{-2} s^{-1}]. Means ±SE represent four replicates. Means are not significantly different if followed by the same superscript letter at the experiment error rate of $p = 0.05$.

	Time of irradiation				
	1 h	3 h	6 h	12 h	18 h
Dark-adapted variable fluorescence yield ($F_{V(M)}$)	151 ± 6^{a}	662 ± 18^{b}	878 ± 20^{c}	$1\,131 \pm 21^{d}$	$1\,037 \pm 25^{d}$
Dark-adapted quantum efficiency of PS2 ($F_{V(M)}/F_M$)	0.23 ± 0.02^{a}	0.68 ± 0.02^{b}	0.79 ± 0.01^{c}	0.83 ± 0.01^{d}	0.85 ± 0.00^{d}
Non-photochemical quenching coefficient (NPQ)	0.12 ± 0.01^{a}	0.37 ± 0.01^{b}	0.36 ± 0.02^{b}	0.73 ± 0.08^{c}	0.41 ± 0.06^{b}
Non-photochemical quenching coefficient (q_N)	0.48 ± 0.03^{a}	0.40 ± 0.01^{ab}	0.34 ± 0.02^{b}	0.50 ± 0.04^{a}	0.34 ± 0.03^{b}
Efficiency of excitation capture by PS2 centers (F'_V/F'_M)	0.15 ± 0.02^{a}	0.56 ± 0.02^{b}	0.69 ± 0.01^{c}	0.71 ± 0.02^{c}	0.78 ± 0.01^{d}
Light-adapted quantum efficiency of PS2 (Φ_{PS2})	0.10 ± 0.01^{a}	0.47 ± 0.02^{b}	0.59 ± 0.01^{cd}	0.54 ± 0.04^{bc}	0.65 ± 0.01^{d}
Photochemical quenching coefficient (q_P)	0.76 ± 0.02^{a}	0.85 ± 0.01^{a}	0.84 ± 0.01^{a}	0.76 ± 0.05^{a}	0.83 ± 0.01^{a}

grown plants that contain functional PS2 centers and non-photochemical dissipation by PS2 centers becomes much less important relative to thermal dissipation by the antennae pigment-protein complex. Our results also parallel those of Jahns and Krause (1994) and Chow *et al.* (2000) who demonstrated that thermal dissipation from the antennae pigment-protein complex is a significant mechanism of excitation-energy dissipation during continuous irradiation-induced chloroplast morphogenesis from intermittent light-grown plants containing functional PS2 centers. The results of this study of excitation energy dissipation during the development of PS2 photochemistry in sunflower etioplasts that initially lacked PS2 suggest that the non-photochemical dissipation results largely from PS2 centers during the earliest stages of the development of PS2 photochemistry. Our results also indicate that thermal dissipation is enhanced during the development of the maximum photochemical potential of PS2 and that changes of mechanisms and magnitudes of non-photochemical dissipation help maintain an optimum fraction of the oxidized state of Q_A during the development of PS2 photochemistry.

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