S3-012

Very high light resistant mutants of *Chlamydomonas reinhardtii* with impaired PSII function: D1 protein dynamics, functional PSII and PSI centers and intersystem electron transport capacities

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Keywords: *Chlamydomonas*, D1 protein, photosystem II function, photosynthetic electron transport, very high light resistance

Introduction

The proportion of light energy utilized in photochemistry and dissipated through nonphotochemical, photoprotective processes (Niyogi, 1999) determines photosystem (PSII) efficiency (Krause and Weis, 1991). The decrease in PSII efficiency (F_v/F_m) observed during exposure of plants to very high light intensities is due either to enhanced photoprotection and/or impaired PSII function and potentially photodamage. Very high light resistant mutants (*VHL*^{R}) of the chlorophyte *Chlamydomonas reinhardtii* grow photoautotrophically in continuous, for other genotypes lethal, VHL (1500-2000 µmol photons m^{-2} s^{-1}) at the same rates as their parental strains in HL (µmol photons m^{-2} s^{-1}) in spite of PSII dysfunction (reduced F_v/F_m) (Förster et al., 1999). *VHL*^{R} mutants arise from the herbicide resistant, PSII/D1 impaired strain *L** with the A251L substitution in the D1 protein (Förster et al., 1997) as readily as from wildtype CC-125. The A251L mutation slowed PSII electron transfer (τ) and eliminated lateral electron transfer between PSII centers (connectivity) at all light intensities (Lardans et al., 1998). Under VHL, τ was even slower in *L** derived *VHL*^{R} mutants (i.e. *L4*, *L30*), whereas wildtype derived mutants (i.e. *S9*, *S4*) primarily lost connectivity (Förster et al., 2001). Clearly, impaired PSII function does not preclude selection or survival of *VHL*^{R} mutants. In this paper we report on relationships between D1 protein turnover, PSII function and intersystem electron transport to photosystem I (PSI) in both classes of *VHL*^{R} mutants grown under high light stress.

Material and methods

C. reinhardtii wildtype CC-125 (WT) was obtained from the Chlamydomonas Genetics Center (Dr. E. Harris, Duke University, USA). The *L** parent strain and *VHL*^{R} mutants were isolated as reported by Förster et al. (1999). Cells were grown in liquid minimal HS media, agitated and bubbled with 5% CO_2 enriched air under low light (LL, 70 µmol photons m^{-2} s^{-1}) HL, VHL or transferred from HL to VHL (H>V) (Förster, 2001). Amounts of D1 protein and light harvesting complex proteins (LHC) were immunodetected in total protein extracts

(Lardans et al., 1997). D1 protein synthesis rates were deduced from 20 min. incorporation of [^{35}S] into protein in sulfate-starved cells. Half-life times of degradation ($t_{1/2}$) were calculated from loss of labeled protein in the presence of the protein synthesis inhibitors anisomycin and lincomycin (Lers et al., 1992). Chlorophyll fluorescence was measured with a PAM fluorometer (Heinz-Waltz, Germany). Amounts of functional PSII centers were estimated from O_2 flash yields using a modified Hansatech O_2 measuring system fitted with white strobe lights (Johnson, 2000). Redox kinetics and amounts of active PSI centers were estimated from far red light (715 ± 15 nm) induced absorbance changes at 800-830 nm using the PAM fluorometer equipped with an ED800-T emitter-detector unit (Asada et al., 1993). Total amounts of chlorophyll (Chl *a*+*b*) were determined in 80% acetone extracts (Porra et al., 1989).

Results and discussion

Pool sizes of D1 protein in *VHL*^{R} mutants and parent strains reflected the kinetics of D1 synthesis and degradation. Technically it was not feasible to block protein degradation without disturbing synthesis. Consequently, D1 synthesis rates represent the balance of simultaneous label incorporation and loss during turnover of the D1 protein. Ratios of synthesis rates corresponded closely to the ratios of pool sizes (Table I). In HL, ratios near 1 indicated that *VHL*^{R} mutants did not differ from parents in HL, but WT derived *S9* grown under VHL showed twice the D1 synthesis rate and pool size as WT exposed to 20 h VHL (Table I). The *L** derived *VHL*^{R} mutants *L4* and *L30*, combined because of their almost identical physiological properties, were relatively unresponsive in pool sizes and synthesis rates to VHL (Förster, 2001).

The $t_{1/2}$ for D1 degradation in HL was similar for both *VHL*^{R} mutants *S9* and *L4* and faster than for their parents (Table II). Transfer of HL grown WT to VHL for 20 h led to 50% faster D1 degradation, consistent with a > 80% decrease in pool size (Förster, 2001), whereas H>V transfer of *S9* initially had no effect on $t_{1/2}$. Continuous growth in VHL, however, slowed D1 degradation in *S9*, partly explaining the larger D1 pool in *S9* relative to WT. In contrast, *L4* retained equally fast D1 degradation under HL and VHL, which may be a partial cause for the same pool sizes in both *L** and *L4* (Tables I, II).

Table I. Ratios of D1 protein pool sizes (normalized to LL) and synthesis rates. Cells were examined in growth light or after 20 h transfer from HL to VHL. [a] data from mutant *L30*. ND= not determined.

Strains		D1 pool	D1 synthesis
S9/WT	HL	1.1	1.0 ±0.0
S9/WT	H>V	2.0	2.2
L4/*L**	HL	[a]1.0	1.2 ±0.0
L4/*L**	H>V	[a]1.0	ND
S9/*L4*	VHL	ND	0.8 ±0.0

Table II. D1 protein degradation ($t_{1/2}$). Cells were examined in growth light intensities or after 20 h transfer from HL to VHL (H>V).

Strain		D1 $t_{1/2}$ (h)
WT	HL	3.5 ± 0.3
	H>V	1.8 ± 0.1
	HL	1.9 ± 0.2
	H>V	1.6 ± 0.1
	VHL	3.4 ± 0.3
L4	HL	2.6 ± 0.3
	HL	1.7 ± 0.2
	VHL	1.8 ± 0.2

Amounts of functional (i.e. O_2 evolving) PSII and maximum efficiencies (F_v/F_m) were similar in LL or HL grown *VHL*^{R} mutants to the respective parents (Table III). During exposure to VHL, a relative loss of functional PSII was more evident in parental strains, whereas *VHL*^{R} mutants retained higher absolute amounts of active PSII. The larger decline in

active PSII centers in WT versus *S9* was consistent with accelerated D1 degradation and decreased synthesis and pool size in WT as opposed to longer $t_{1/2}$ and larger D1 pool size in the *VHL*^{R} mutant (Tables II and I). Unlike A251L mutants, *S9* maintained fast forward PSII electron transfer although connectivity was lost under high light stress (Förster et al., 2001). Under most growth light intensities, *L4* and *L** showed lower F_v/F_m and fewer active PSII centers than WT and *S9* (Table III) as a consequence of impaired PSII electron transfer due to the A251L mutation. Because D1 synthesis in *L4* exceeded that of *S9* under VHL (Table I), and because effects on the D1 pool size or $t_{1/2}$ in *L** and *L4* (Table II) were marginal in HL and VHL, we conclude that a higher proportion of inactive/impaired PSII centers was retained (i.e. more D1 protein) in the A251L mutants. Even though *L4* appeared to survive with less change in D1 turnover than *S9* under 1500 µmol photons m^{-2} s^{-1}, perhaps indicative of less photodamage, the *VHL*^{R} and D1 double mutant was not viable under 2000 µmol photons m^{-2} s^{-1} (Förster et al., 2001).

As with PSII, amounts of active PSI and function (P700 oxidation rates) declined more strongly in WT under HL and VHL (Table III) than in the *VHL*^{R} mutant *S9*. Actually, both *S9* and *L4* maintained higher amounts and function of PSI centers in VHL than their parent strains, and showed even faster P700 oxidation than in LL. The A251L mutation was associated with reduced adaptation of photosystems to high light stress, particularly evident from in *L4*. *VHL*^{R} mutants and *L** had generally higher chlorophyll contents than WT. Chl concentrations decreased in all strains with higher growth light intensities, indicative of smaller and/or fewer antennae, which was approximately reflected in diminished amounts of LHC protein. *L4* responded to VHL with relatively less reduction in Chl than *S9*. Altogether, survival under VHL appears to involve down-sizing of the light capturing capacity of the photosynthetic apparatus to relieve excitation pressure.

Table III. PSII efficiency F_v/F_m, amounts of functional PSII and PSI centers normalized to biomass (A_{750}), rates of P700 oxidation estimated from far red induced absorbance changes at 800-830 nm, total chlorophyll per biomass and amounts of LHC proteins (normalized to respective LL grown parents).
[a] determined from mutant *L30*.

Strains		**F_v/F_m**	**PSII** pmol A_{750}^{-1}		**PSI** µV A_{750}^{-1}		**P700$_{ox}$** µV s^{-1}		**Chl** µg A_{750}^{-1}	**LHC** (%)
	LL	0.72	53	±6	120	±2	142	± 6	38 ±3	100
	HL	0.74	39	±4	57	±2	115	± 8	23 ±2	50
	H>V	0.48	19	±1	10	±0	30	± 2	11	1
S9	LL	0.72	46	±6	112	±5	133	± 7	48 ±3	104
	HL	0.69	48	±6	58	±1	114	± 5	30 ±2	56
	VHL	0.57	30	±4	26	±0	145	± 4	16 ±1	22
L*	LL	0.54	33	±3	79	±4	101	± 5	41 ±2	100
	HL	0.63	36	±5	72	±3	123	± 8	36 ±2	74
	H>V	0.41	22	±3	38	±4	93	± 8	19	45
L4	LL	0.41	31	±1	58	±0	114	± 5	44 ±3	[a] 75
	HL	0.54	29	±1	78	±1	226	± 9	32 ±1	[a] 62
	VHL	0.37	30	±1	53	±1	179	±18	22 ±1	[a] 37

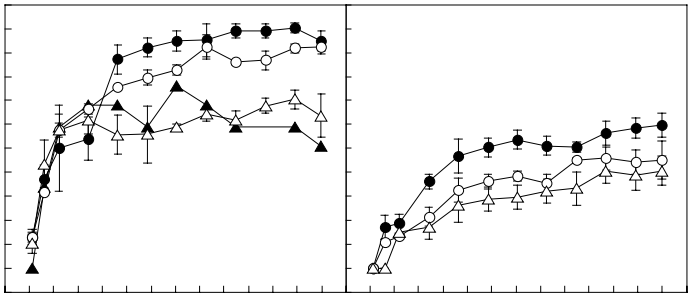

Fig. 1. Re-reduction of far-red induced $P700^+$ by red light. HL grown (circles) and VHL exposed (triangles) parents (filled symbols) and VHL^R mutants (open symbols). Left panel: WT and *S9*. Right panel: L^* and *L4*.

Far-red induced $P700^+$ formation (Figure 1) is reversed by red light, reflecting electron delivery from PSII via intersystem carriers to PSI. In WT and *S9* 150 µmol photons m^{-2} s^{-1} red light re-reduced 50% of $P700^+$ in both HL and VHL exposed cells, but maximum relative $P700^+$reversal was less in VHL cells. As high as 1200 µmol photons m^{-2} s^{-1} red light were insufficient to re-reduce $P700^+$ more than 45-60% in *L4* and L^* exposed to HL or VHL both of which had 3-5 fold slower PSII electron transfer. In fact, VHL exposed L^* lacked red-reversal completely. These observations are consistent with the notion that slow PSII electron transfer prevents the generation of sufficient ΔpH to transform a high de-epoxidation state of the xanthophyll pigments into nonphoto-chemical quenching (Förster et al., 2001). They are also consistent with the proposition that antennae downsizing and/or photodamage in *L4* may be less than in *S9* because slow PSII electron transfer precludes the generation of regulatory signals and/or oxidants to the same extent that may occur in *S9* under VHL.

Acknowledgements

The authors are grateful for intellectual contributions of Peter Heifetz and steady guidance of Nick Gillham. Supported by DOE grant DE-FG05-89ER14005.

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