

Photosystem II Excitation Pressure and Development of Resistance to Photoinhibition¹

I. Light-Harvesting Complex II Abundance and Zeaxanthin Content in *Chlorella vulgaris*

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The basis of the increased resistance to photoinhibition upon growth at low temperature was investigated. Photosystem II (PSII) excitation pressure was estimated in vivo as $1 - q_p$ (photochemical quenching). We established that *Chlorella vulgaris* exposed to either 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ experienced a high PSII excitation pressure of 0.70 to 0.75. In contrast, *Chlorella* exposed to either 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or 5°C/20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ experienced a low PSII excitation pressure of 0.10 to 0.20. *Chlorella* grown under either regime at high PSII excitation pressure exhibited: (a) 3-fold higher light-saturated rates of O_2 evolution; (b) the complete conversion of PSII α centers to PSII β centers; (c) a 3-fold lower epoxidation state of the xanthophyll cycle intermediates; (d) a 2.4-fold higher ratio of chlorophyll *a/b*; and (e) a lower abundance of light-harvesting polypeptides than *Chlorella* grown at either regime at low PSII excitation pressure. In addition, cells grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ exhibited resistance to photoinhibition comparable to that of cells grown at 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and were 3- to 4-fold more resistant to photoinhibition than cells grown at either regime at low excitation pressure. We conclude that increased resistance to photoinhibition upon growth at low temperature reflects photosynthetic adjustment to high excitation pressure, which results in an increased capacity for nonradiative dissipation of excess light through zeaxanthin coupled with a lower probability of light absorption due to reduced chlorophyll per cell and decreased abundance of light-harvesting polypeptides.

In studies that attempt to delineate the mechanism(s) of photosynthetic adjustment to temperature, one typically compares the structure and function of the photosynthetic apparatus of plants or algae grown to the same developmental state at a high temperature with those grown at a low temperature but at constant irradiance and photoperiod. Thus, one assumes that any alterations in the structure and function of the photosynthetic apparatus reflect a direct response to growth temperature (Huner, 1985, 1988; Huner et al., 1993). However, recently, through a study in which *Chlorella vulgaris* grown under continuous light at

5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was compared to *C. vulgaris* grown at 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, we concluded that this assumption is invalid (Maxwell et al., 1994). Based on published data concerning acclimation of algae to high irradiance (Sukenic et al., 1988; Senge and Senger, 1990; Smith et al., 1990; Harrison et al., 1992), we suggested that growth of *C. vulgaris* at low temperature mimics high-light acclimation even though *C. vulgaris* was grown at a low to moderate irradiance of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Maxwell et al., 1994). For example, compared to growth at 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, *Chlorella* grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ exhibited a doubling in the light-saturated rate of CO_2 -saturated O_2 evolution, a 2-fold increase in the ratio of Chl *a/b*, a 5-fold lower total Chl per cell, decreased abundance of LHClI polypeptides, and increased total xanthophyll content (Maxwell et al., 1994). However, differences in pigment content and composition were eliminated either by reducing the growth irradiance at 5°C from 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or by keeping the irradiance constant at 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and shifting the cells from 5°C to 27°C for 12 h. Thus, we suggested that, due to the thermodynamic constraints, *C. vulgaris* grown at 5°C does not respond to growth temperature per se but rather to increased excitation pressure on PSII. Thus, we surmised that *Chlorella* can be released from the constraints of excessive PSII excitation pressure either by maintaining a constant temperature and decreasing the irradiance or, alternatively, by maintaining constant irradiance and increasing the temperature.

It has been established that growth of spinach (Somersalo and Krause, 1989, 1990; Boese and Huner, 1990; Gray et al., 1994), rye (Öquist and Huner, 1992), wheat (Hurry and Huner, 1992; Öquist et al., 1993), and *Chlamydomonas reinhardtii* (Falk et al., 1990) at low temperature and moderate irradiance (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$) results in resistance to

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Abbreviations: EPS, epoxidation state (violaxanthin + 0.5 antheraxanthin)/(violaxanthin + antheraxanthin + zeaxanthin); F_M and F_O , fluorescence when all dark-acclimated PSII reaction centers are closed and open, respectively; F_V , variable fluorescence after dark acclimation ($F_M - F_O$); F_V/F_M , the ratio of variable to maximum fluorescence as an expression of the maximum photochemical yield of PSII in the dark-acclimated state; LHClI, the major Chl *a/b* light-harvesting complex of PSII; Q_A , the primary, stable, quinone electron acceptor of PSII; q_p , photochemical quenching; Z, zeaxanthin.

light 5- to 6-fold higher than the growth irradiance. Growth at low temperature is a prerequisite for the acquisition of this resistance to photoinhibition (Gray et al., 1994). The goal of this study was to elucidate the basis of this response. We hypothesized that differential resistance to photoinhibition observed between plants and algae grown at low or high temperature is not due to growth temperature per se but to growth under differential PSII excitation pressures. In this report, we test this hypothesis by addressing the following questions: (a) Is *Chlorella* grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically comparable to *Chlorella* grown at 27°C and high light, i.e. high PSII excitation pressure? Alternatively, is *Chlorella* grown at 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically comparable to *Chlorella* grown at 5°C and low light, i.e. low excitation pressure? (b) If so, does *Chlorella* grown under comparable PSII excitation pressure exhibit comparable resistance to photoinhibition?

MATERIALS AND METHODS

Plant Material and Culture Conditions

The unicellular green alga *Chlorella vulgaris* Beijer. (University of Texas Culture Collection strain UTEX 265) was grown axenically in Bold's basal medium as previously described (Maxwell et al., 1994). All experiments were conducted using cells grown in 150-mL Pyrex glass tubes immersed in aquaria with the thermostat set at either $27 \pm 1^\circ\text{C}$ (27°C cells) or $5 \pm 1^\circ\text{C}$ (5°C cells). Each culture tube was bubbled with sterile air (150 mL/min) and exposed to an irradiance of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplied by fluorescent tubes (Sylvania CW-40). Low-temperature, low-light cells were exposed to an irradiance of 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ by using the same light source in combination with a neutral density filter. High-temperature, high-light cells were grown at 2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using a 300-W halogen lamp (General Electric). The irradiance was measured from the middle of each culture tube using a quantum sensor (model 185A, Li-Cor, Lincoln, NE). Cultures were maintained at low Chl concentrations (2–5 $\mu\text{g mL}^{-1}$) and in the exponential growth phase by dilution with fresh medium.

Photoinhibition Treatment

All photoinhibitory treatments were performed using cells contained in the same glass tubes and aquaria used for normal cultivation. High irradiance was generated using a 500-W halogen lamp (General Electric) placed adjacent to the aquaria, with the irradiance measured from within the culture tube as described above. Photoinhibition was assayed using an irradiance of 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for a maximum of 2 h at 5°C.

Pigment Extraction

Pigments were extracted into 90% (v/v) acetone and quantified as previously described (Maxwell et al., 1994). For HPLC analysis pigments were extracted into 100%

HPLC-grade acetone (BDH, Toronto, Ontario, Canada) and subsequently passed through a 2- μm syringe filter before being stored under nitrogen at -20°C .

HPLC

Photosynthetic pigments were separated by HPLC using a Beckman System Gold solvent module (Beckman Instruments, San Ramon, CA) equipped with a Spherisorb ODS-1 analytical column (5- μm particle size, 250 mm \times 4.6 mm i.d.), which was protected by an Upchurch Perisorb A guard column (both columns from S.P.E. Inc., Concord, Ontario, Canada). Samples were injected using a Beckman 210A sample-injection valve with a 20- μL sample loop. Through modifications of the method of Gilmore and Yamamoto (1991) pigments were eluted using 100% acetonitrile:methanol:0.1 M Tris (pH 8.0) (74:11:3.5) followed by a 2-min linear gradient to 100% methanol:hexane (4:1), which continued isocratically until the end of the 12-min separation. The column was allowed to re-equilibrate in the initial solvent for a minimum of 10 min between injections. All solvents were of HPLC grade (Omni Solv, BDH).

Pigments were detected with a Beckman System Gold diode array detector at 440 nm and peak areas were integrated by Beckman System Gold software. Retention times and response factors were determined by injecting known quantities of pure Chl *a*, Chl *b*, lutein, and β -carotene (Sigma). The retention times of Z, antheraxanthin, violaxanthin, and neoxanthin were determined by using pigments purified from barley according to Diaz et al. (1990).

Measurement of Excitation Pressure

Excitation pressure on PSII reflects the proportion of Q_A in the reduced state, $(Q_A)_{\text{red}}/[(Q_A)_{\text{red}} + (Q_A)_{\text{ox}}]$. It is calculated as $1 - q_P$ (Demmig-Adams et al., 1990), where q_P is the coefficient of photochemical quenching (van Kooten and Snel, 1990). All measurements were made using the pulse amplitude modulated fluorescence system as previously described (Maxwell et al., 1994).

Measurements of PSII Heterogeneity

Algal samples were transferred to 1.5-mL microcentrifuge tubes and dark adapted for 5 min with or without addition of 10 μM DCMU. The recorded signals of fluorescence from DCMU-poisoned cells were used to calculate the amount of PSII α and PSII β centers according to Melis and Homann (1975, 1976). The room temperature fluorescence signal was generated using a Plant Stress Meter (PSM, BioMonitor, SCI AB, Umea, Sweden) with an actinic light of 60 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for DCMU-poisoned and nonpoisoned samples, respectively. The fluorescence signals were recorded and stored on a computer hard disk using an oscilloscope card (PC-SCOPE T6420, Intelligent Messtechnik GmbH, Backnang, Germany) with a sampling frequency of 10 kHz. Fluorescence traces consisted of 2950 data pairs recorded during the first 3 s of illumination and were fitted to a model consisting of two exponentials as

described in detail by Falk et al. (1994):

$$y = a(1 - e^{-bx}) = c(1 - e^{-dx}) + k \quad (1)$$

where k is the y -intercept, a and c are the variable fluorescence amplitudes, and b and d are the relative growth rates of each exponential using the SigmaPlot 5.0 (Jandel Scientific, San Rafael, CA) nonlinear curve fitting by the Levenberg-Marquardt method of nonlinear least squares. The resulting values for each parameter were used to calculate the proportion of the PSII β component (Falk et al., 1994) and the proportion of PSII α was calculated as $1 - \text{PSII}\beta$.

Thylakoid Membrane Isolation and SDS-PAGE

Thylakoid membranes were isolated as previously described (Maxwell et al., 1994). SDS-PAGE was performed using a Mini-protein II apparatus (Bio-Rad), a 12% (w/v) polyacrylamide separating gel, and the buffer system of Laemmli (1970).

Western Blots

Membrane polypeptides separated as above were electrophoretically transferred to Immobilon (Millipore, Bedford, MA) according to Tsang et al. (1983) and probed with polyclonal antibodies raised against spinach LHClI (M. Krol, M.D. Spangfort, N.P.A. Huner, G. Öquist, P. Gustafsson, S. Jansson, unpublished data). Blots were developed using alkaline phosphatase coupled to goat anti-rabbit IgG (Sigma) according to Dunn (1986).

Measurements of Photosynthesis

Measurements of oxygen evolution using an aqueous-phase Hansatech Ltd. (King's Lynn, UK) O₂ electrode and steady-state Chl *a* fluorescence using a pulse amplitude modulated Chl fluorometer (H. Walz, Effeltrich, Germany) were performed as described in detail elsewhere (Maxwell et al., 1994).

Protein Determination

The protein concentration of isolated thylakoid membranes was determined using the bicinchoninic acid method (Pierce, Rockford, IL). Chl was removed by resuspending membrane samples (5–10 μL) in 1 mL of 90% (v/v) acetone followed by centrifugation at 12,000g for 5 min to sediment the protein. After allowing the pellet to air dry it was solubilized in 100 μL of 2% (w/v) SDS and heated at 60°C for 30 min. Protein content was determined according to the instructions provided using the standard incubation protocol of 30 min at 37°C.

RESULTS

Effect of Temperature on PSII Excitation Pressure

Q_A is reduced by incident irradiance and subsequently oxidized by intersystem electron transport. The rates of the photochemical reactions of charge separation are essentially insensitive to temperature in the biologically relevant

range (0–50°C) and exceed those of thermochemical processes such as intersystem electron transport and CO₂ fixation. Thus, modulation of excitation pressure on PSII by increasing incident irradiance at constant temperature should be equivalent to decreasing the temperature at constant irradiance. This is illustrated by the results in Figure 1 for *Chlorella* grown at 27°C and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At a constant measuring temperature of 27°C (open circles), excitation pressure on PSII ($1 - q_p$) increased with increasing irradiance. As expected, if the measuring temperature was decreased to 5°C (filled circles), excitation pressure on PSII was higher at all light levels due to thermodynamic constraints on intersystem electron transport and CO₂ fixation. Furthermore, the data in Figure 1 indicate that exposure of 27°C-grown *Chlorella* to a measuring temperature of 5°C at the same irradiance of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ results in a $1 - q_p$ of 0.70 to 0.75, which is comparable to exposure of the 27°C cells to about 2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at a measuring temperature of 27°C. From these data we hypothesized that *Chlorella* cells grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ should be photosynthetically comparable to cells grown at 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ because they would experience a similar, high PSII excitation pressure. Similarly, *Chlorella* grown at 5°C/20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ should be photosynthetically comparable to cells grown at 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ because they would experience a similar, low PSII excitation pressure (0.10–0.20).

Photosynthetic O₂ Evolution and Chl *a* Fluorescence Induction

As shown in Figure 2, *Chlorella* exposed to either growth regime at low PSII excitation pressure (Fig. 2, open and filled circles) exhibited comparable light response curves for CO₂-saturated O₂ evolution. A similar trend was observed for cells exposed to either growth regime at high excitation pressure (Fig. 2, open and filled squares). Furthermore, both populations of *Chlorella* grown at high excitation pressure exhibited light-saturated rates of photosynthesis that were 3-fold higher than either population grown at low PSII excitation pressure. Although Figure 2

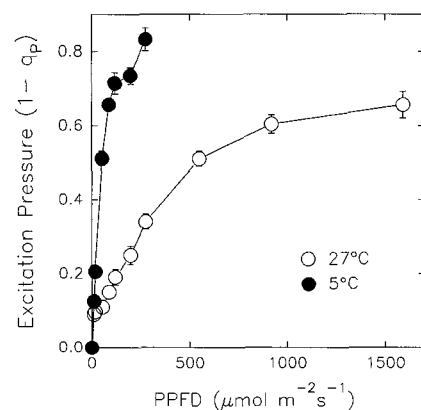


Figure 1. Excitation pressure on PSII as a function of measuring irradiance for cells grown at 27°C and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements were performed at 5°C (●) and 27°C (○).

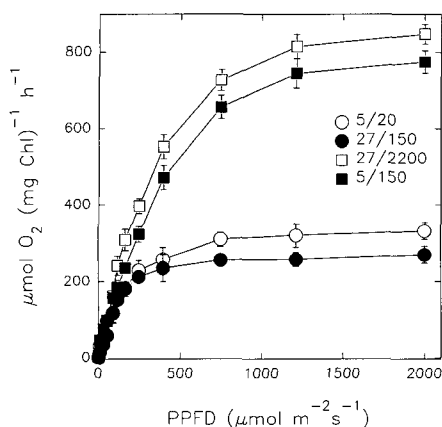


Figure 2. Light-response curves of O_2 evolution of *C. vulgaris* grown under different temperature and irradiance conditions but measured at 27°C. Measurements are presented as gross O_2 evolution. Bars represent SE; $n = 3$ to 4.

illustrates results obtained at a measuring temperature of 27°C, similar trends in the light-response curves were observed at a measuring temperature of 5°C (data not shown), which is consistent with previous results (Maxwell et al., 1994).

The results for O_2 evolution were corroborated by in vivo Chl *a* fluorescence (Fig. 3). Cells grown under both growth regimes that elicit high excitation pressure exhibited similar fluorescence induction curves but were characterized by a lower variable fluorescence than cells exposed to either growth regime at low PSII excitation pressure. This was also reflected in a 15% lower F_V/F_M in cells grown at high compared to low PSII excitation pressure (Table I). A more detailed analysis of the fast fluorescence transients in the presence or absence of DCMU indicated that PSII α centers were absent in cells exposed to either growth regime at high PSII excitation pressure, whereas PSII α :PSII β was approximately 1:2 in cells exposed to either growth regime at low excitation pressure (Table I).

Pigment Composition and LHCII Abundance

As shown in Table II, the pigment composition of *Chlorella* grown at either 5°C/20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ exhibited high xanthophyll EPS's and a Chl *a/b* of about 3.6. In contrast, cells grown at either 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ exhibited low EPS's and high Chl *a/b* ratios expected for cells grown under high excitation pressure. Pigment analyses for isolated *Chlorella* thylakoids (data not shown) were similar to those presented for intact cells (Table II). However, we note that Z levels were 2-fold higher in control cells grown at 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ than in control cells grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table II). This is consistent with higher xanthophyll cycle activity at 27°C than at 5°C.

Consistent with the changes in Chl *a/b* (Table II), the Coomassie blue-stained SDS polyacrylamide gels as well as the western blots (Fig. 4) illustrate that LHCII abundance was also sensitive to PSII excitation pressure experienced during growth of *Chlorella*. Both cell populations grown

under high PSII excitation pressure exhibited similar levels of LHCII but lower LHCII abundance than for either *Chlorella* population grown at low excitation pressure. The lower LHCII abundance in cells grown at high excitation pressure was associated with a doubling in the ratio of protein:Chl and a 5-fold lower total Chl per cell (Table I). Thus, the results for O_2 evolution, Chl *a* fluorescence, pigment content and composition, and LHCII abundance support our contention that *Chlorella* grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are photosynthetically similar because they experienced comparable PSII excitation pressure. For the same reason, *Chlorella* grown at 5°C/20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are photosynthetically similar to cells grown at 27°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Role of Excitation Pressure on Resistance to Photoinhibition

Since *Chlorella* cells grown at 5°C/150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were photosynthetically comparable to cells grown at 27°C/2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, we assessed the susceptibility of both populations of cells to low-temperature photoinhibition. Figure 5E illustrates that the maximal photochemical efficiency (F_V/F_M) of both populations of cells grown under high excitation pressure exhibited comparable sensitivity to low-temperature photoinhibition. Furthermore, F_V/F_M of *Chlorella* grown under high excitation pressure (Fig. 5E) was less sensitive to low-temperature photoinhibition than cells grown under low excitation pressure (Fig. 5F). This trend was observed whether photoinhibition was performed at 5 or 27°C (D.P. Maxwell and N.P.A. Huner, unpublished data) and was consistent for measurements of light response curves for O_2 evolution before and after photoinhibition (Maxwell et al., 1993). In addition, cells grown under high excitation pressure exhibited, first, a higher initial rate as well as a greater extent of F_0 quenching (Fig. 5, C and D). In contrast, cells grown at low excitation pressure exhibited an initial increase in F_0 followed by quenching of F_0 fluorescence. Furthermore, the

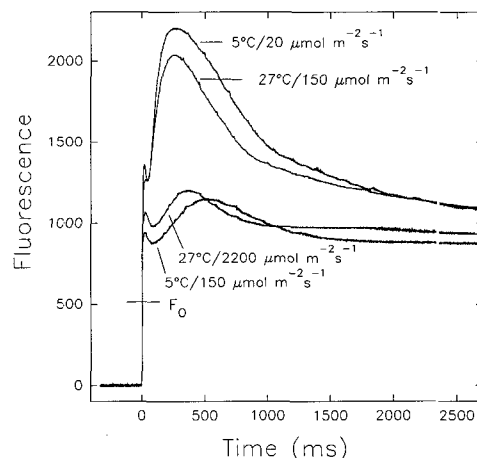


Figure 3. Representative room temperature fluorescence induction curves of *C. vulgaris* for two growth conditions each of high and low excitation pressure.

Table I. Characteristics of *C. vulgaris* grown under two conditions each of high and low excitation pressureValues represent means \pm SD, $n = 3$ to 6. Calculation of PSII α and PSII β represent the results of a single experiment.

Characteristic	Growth Temperature ($^{\circ}$ C)/Growth Irradiance (μ mol m^{-2} s^{-1})			
	27/150	5/20	5/150	27/2200
F_V/F_M	0.741 \pm 0.013	0.752 \pm 0.014	0.695 \pm 0.023	0.611 \pm 0.019
fg Chl/cell	730 \pm 41	716 \pm 38	130 \pm 15	180 \pm 22
Membrane protein/Chl (mg/mg)	4.52 \pm 0.23	4.35 \pm 0.32	9.0 \pm 0.32	8.4 \pm 0.25
Percent of PSII α	31.1	30.0	0	0
Percent of PSII β	68.9	70.0	100	100

final extent of F_O quenching was greater in those cells exposed to high excitation pressure than in those grown at low excitation pressure. Second, *Chlorella* grown at high excitation pressure exhibited a reduced level of F_V quenching compared to *Chlorella* grown at low excitation pressure (Fig. 5, A and B). Thus, as expected, cells grown under equivalent excitation pressure exhibit comparable sensitivity to photoinhibition.

However, we note that *Chlorella* grown at 5° C is still about 15% more resistant to photoinhibition than 27° C-grown cells regardless of PSII excitation pressures (compare Fig. 5, E and F). At high excitation pressures, this growth temperature effect appears to be due to decreased F_V quenching (Fig. 5A), whereas at low excitation pressures, this growth temperature effect appears to be due to a decreased stimulation of F_O fluorescence upon initial exposure to photoinhibitory light (Fig. 5D).

Role of Z in the Quenching of F_O

Prior to exposure to low-temperature photoinhibition, growth of *Chlorella* at either treatment of high PSII excitation pressure (5° C/ 150μ mol m^{-2} s^{-1} and 27° C/ 2200μ mol m^{-2} s^{-1}) resulted in a 16- to 32-fold higher level of Z and consequently a lower EPS than cells grown at either treatment at low excitation pressure (5° C/ 20μ mol m^{-2} s^{-1} and 27° C/ 150μ mol m^{-2} s^{-1}) (Table II). Upon exposure to photoinhibition at 5° C, both populations of cells grown at low excitation pressure exhibited a 10-fold increase in the level of Z from about 5 to 50 mmol Z mol^{-1} Chl *a* (Table II),

whereas cells grown at high excitation pressure exhibited a 1.2- to 2.0-fold increase in Z. However, the absolute levels of Z were almost 4-fold higher after photoinhibition in cells grown at high excitation pressure and as a consequence exhibited lower EPS's than cells grown at low excitation pressure (Table II).

Figure 6 illustrates the kinetics of Z accumulation during photoinhibition in cells grown at high (filled symbols) and low excitation pressures (open symbols). The rapid initial quenching of F_O in cells grown at high excitation pressure (Fig. 5C) is correlated with a high initial level of Z coupled with a rapid increase in total Z (Fig. 6, filled symbols). In contrast, the initial 25% increase in F_O in cells grown at low excitation pressure at 27° C (Fig. 5D) is correlated with an extremely low initial level of Z coupled with a lag of about 15 min before maximum rates of Z accumulation were attained (Fig. 6, open symbols). Subsequently, F_O was quenched to at least the initial level. Thus, the greater resistance to photoinhibition observed in *Chlorella* grown at high excitation pressure may occur, in part, as a consequence of increased capacity for nonradiative dissipation of excess light energy through Z (Demmig-Adams, 1990).

DISCUSSION

Based on the results for CO_2 -saturated O_2 evolution (Fig. 2), Chl *a* fluorescence induction (Table I; Fig. 3), pigment content (Table I) and composition (Table II), and LHClI abundance (Fig. 4), *C. vulgaris* grown at low temperature and moderate light (5° C/ 150μ mol m^{-2} s^{-1}) was photo-

Table II. Photosynthetic pigments isolated from whole-cell extracts of *C. vulgaris* grown under two conditions each of high and low excitation pressure

Numbers under Growth Condition indicate growth temperature ($^{\circ}$ C)/growth irradiance (μ mol m^{-2} s^{-1}). Pigments were extracted during steady-state growth or immediately following a 120-min photoinhibitory (PI) assay of 1300μ mol m^{-2} s^{-1} at 5° C. Pigments were separated and quantified by HPLC. All values are relative to Chl *a* (mmol mol^{-1} Chl *a*) except Chl *a*/Chl *b*, which is (mol/mol). Data represent the means \pm SE; $n = 3$ to 5. A, Antheraxanthin; N, neoxanthin; V, violaxanthin.

Growth Condition	Treatment	Pigment Concentration							
		N	V	A	Z	EPS	Lutein	β -Carotene	Chl <i>a</i> /Chl <i>b</i>
27/150	Control	33.9 \pm 3.4	68.2 \pm 4.2	2.9 \pm 0.6	5.9 \pm 0.9	0.905	228.9 \pm 9.8	62.4 \pm 7.4	3.59 \pm 0.08
	Post-PI	29.7 \pm 2.5	11.4 \pm 3.2	9.1 \pm 1.1	54.2 \pm 5.1	0.213	231.5 \pm 16.2	63.5 \pm 5.5	3.63 \pm 0.07
5/15	Control	41.5 \pm 5.3	63.2 \pm 5.8	2.1 \pm 0.8	5.1 \pm 1.2	0.912	268 \pm 11.5	75.5 \pm 8.6	3.61 \pm 0.11
	Post-PI	35.2 \pm 4.5	13.5 \pm 3.4	11.4 \pm 2.5	51.5 \pm 3.5	0.259	278 \pm 9.5	74.2 \pm 3.5	3.54 \pm 0.09
5/150	Control	30.2 \pm 2.8	54.4 \pm 2.7	47.5 \pm 4.3	95.8 \pm 5.5	0.395	394.7 \pm 23.0	120.8 \pm 16.2	8.47 \pm 0.24
	Post-PI	29.7 \pm 2.5	2.1 \pm 0.3	10.3 \pm 1.4	196.5 \pm 11.3	0.035	412.8 \pm 18.2	140.4 \pm 8.2	8.65 \pm 0.42
27/2200	Control	25.9 \pm 5.4	15.8 \pm 3.5	41.6 \pm 8.5	194.5 \pm 17.5	0.145	442.6 \pm 23.6	117.4 \pm 3.9	7.92 \pm 0.58
	Post-PI	32.8 \pm 4.4	6.4 \pm 0.9	12.1 \pm 2.5	241.3 \pm 13.5	0.056	423.2 \pm 15.8	106.2 \pm 14.3	7.75 \pm 0.48

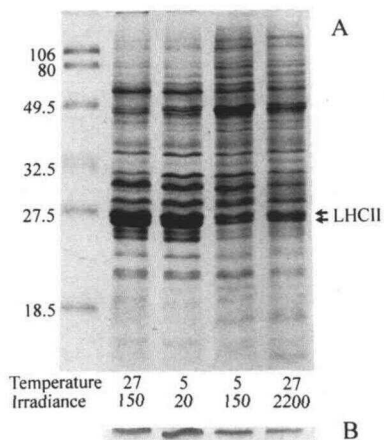


Figure 4. A, SDS-PAGE of membrane polypeptides from *C. vulgaris* grown under two treatments each of high and low excitation pressure. Numbers beneath the gel represent growth temperature ($^{\circ}\text{C}$) and irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Each lane was loaded with $20 \mu\text{g}$ of protein. Numbers at left indicate molecular masses (kD) of the standards in the first lane. B, Western blot analysis of the 26-kD LHCII polypeptide from a gel similar to the one above (see "Materials and Methods").

synthetically comparable to high-light-grown *Chlorella* ($27^{\circ}\text{C}/2200 \mu\text{mol m}^{-2} \text{s}^{-1}$). We suggest that this is due to the fact that, as a consequence of thermodynamic constraints, cells developed at low temperature but moderate irradiance were exposed to a high PSII excitation pressure that was comparable to that experienced by cells grown at high temperature and high light. Similarly, cells grown at low temperature and low light ($5^{\circ}\text{C}/20 \mu\text{mol m}^{-2} \text{s}^{-1}$) were photosynthetically comparable to cells grown at high temperature and moderate light ($27^{\circ}\text{C}/150 \mu\text{mol m}^{-2} \text{s}^{-1}$) because both populations developed under conditions of low PSII excitation pressure. This supports our contention that modulation of PSII excitation pressure by temperature results in alterations in the structure and function of the photosynthetic apparatus that are comparable to modulation of PSII excitation pressure by high light. Recently, Davison and Falkowski (1994) have also suggested that thermal acclimation and photoacclimation may be controlled by the same mechanisms.

Several recent reports have indicated that plants grown at low temperature ($5^{\circ}\text{C}/250 \mu\text{mol m}^{-2} \text{s}^{-1}$) are more resistant to photoinhibition than control plants grown at high temperature ($20^{\circ}\text{C}/250 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Somersalo and Krause, 1989, 1990; Huner et al., 1993). The results presented in this report for photoinhibition in *Chlorella* grown at high or low temperature are consistent with published reports for higher plants. *Chlorella* grown at $5^{\circ}\text{C}/150 \mu\text{mol m}^{-2} \text{s}^{-1}$ were significantly more resistant to photoinhibition than cells grown at $27^{\circ}\text{C}/150 \mu\text{mol m}^{-2} \text{s}^{-1}$. Since these populations of cells have been grown at two different temperatures but constant irradiance, it may be assumed that increased resistance to photoinhibition is a simple growth temperature response. However, this assumption is incorrect, since although both populations have grown at constant irradiance, they have grown under

vastly different PSII excitation pressures. Due to thermodynamic constraints, plants and algae growing at constant irradiance but at low temperature must adjust to a much higher excitation pressure ($1 - q_P = 0.72-0.75$) than those growing at the same irradiance but higher temperature ($1 - q_P = 0.10-0.20$). For this reason, plants or algae grown at low temperature but moderate irradiance exhibit photosynthetic characteristics of plants or algae grown under high light. This, in turn, imparts increased resistance to photoinhibition.

The results presented in this report bring into question the proper control to be used in experiments where one is attempting to elucidate the long-term growth temperature effects or short-term temperature stress effects. Since in most typical experiments of this nature organisms are exposed to light, it is our contention that physiological, biochemical, and molecular alterations should be monitored as a function of irradiance in order to separate temperature effects per se from potential effects due to PSII excitation pressure. For example, the majority of resistance to photoinhibition seen in *Chlorella* grown at 5°C compared to 27°C and the same irradiance is not due to acclimation to low temperature but to acclimation to higher excitation pressure. Only when one compares cells grown at $5^{\circ}\text{C}/150 \mu\text{mol m}^{-2} \text{s}^{-1}$ with those grown at $27^{\circ}\text{C}/2200 \mu\text{mol m}^{-2}$

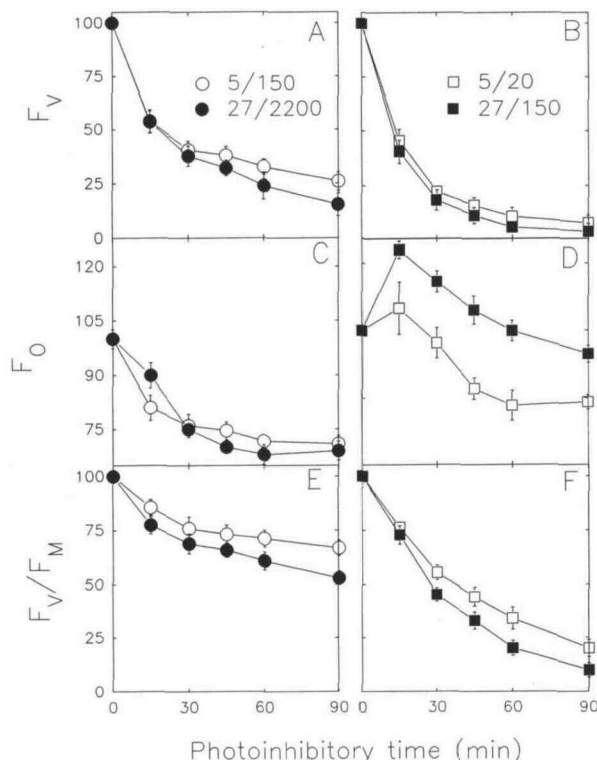


Figure 5. Effect of a short-term (90 min) high-light ($1300 \mu\text{mol m}^{-2} \text{s}^{-1}$) treatment at 5°C on sensitivity to photoinhibition in *C. vulgaris* grown under either high (left panels) or low (right panels) excitation pressure. The high excitation pressure growth regimes were 5°C and $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\circ) or 27°C and $2200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\bullet). The low excitation pressure growth regimes were 5°C and $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\square) or 27°C and $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (\blacksquare). Bars represent SE; $n = 3$ to 5.

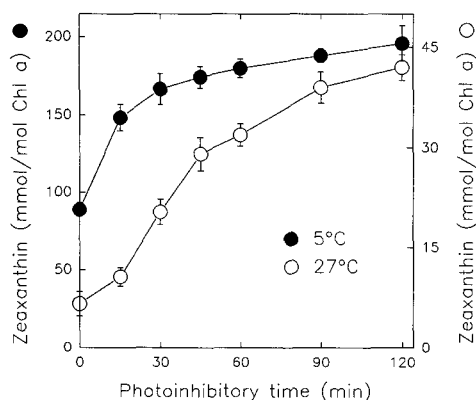


Figure 6. Extent of Z formation as a function of photoinhibitory time in *C. vulgaris* grown at 27°C (○) and 150 $\mu\text{mol}^{-2} \text{s}^{-1}$ or 5°C (●) and the same irradiance. Photoinhibitory protocol as for Figure 5, except 120 min in duration. Bars represent SE; $n = 3$.

s^{-1} (Fig. 6E), i.e. at similar excitation pressures, can we observe that the 5°C cells are still more resistant to photoinhibition than those grown at 27°C. We conclude that this difference must be due solely to growth temperature. This is supported further by the results illustrated in Figure 5F for growth at low excitation pressure.

We believe that the importance of PSII excitation pressure may be extended to other environmental stresses besides low temperature and high irradiance. For example, nutrient stress (Greene et al., 1992) and water stress (Cowan et al., 1992) in algae result in photosynthetic alterations similar to those reported here for growth at low temperature or high irradiance. We suggest that these stresses may ultimately influence excitation pressure through feedback mechanisms on PSII because of their potential to reduce growth rates and rates of metabolism. Therefore, we believe that modulation of excitation pressure is a central component of a general signaling mechanism to initiate appropriate alterations to the photosynthetic apparatus at the physiological, biochemical, and molecular levels in response to environmental change. Recently, we have shown that excitation pressure and not irradiance per se regulates *cab* gene transcript levels in *Dunaliella salina* (D.P. Maxwell, D.E. Laudenbach, and N.P.A. Huner, unpublished data).

Increased levels of Z in the light-harvesting antenna have been implicated to protect PSII upon exposure to excessive light by dissipating excess absorbed light energy as heat in higher plants (Demmig-Adams, 1990) and algae (Franklin et al., 1992). This energy dissipation is often reflected in a quenching of the F_0 level (Weis and Berry, 1987; Demmig-Adams et al., 1988). In addition, lower levels of LHCII associated with PSII may aid in protection against high light (Cleland and Melis, 1987; Mäenpää et al., 1987). Thus, we suggest that the increased resistance to photoinhibition observed in *Chlorella* grown at 5°C/150 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ is due in part to an increased capacity for nonradiative dissipation of excess light through Z combined with a reduced capacity to absorb light as a consequence of lower Chl per cell and lower levels of LHCII.

In conclusion, our results for O_2 evolution, Chl *a* fluorescence, pigment composition, and LHCII abundance indicate that modulation of PSII excitation pressure by either low temperature or high light can result in comparable alterations in the structure and function of the photosynthetic apparatus of *Chlorella*. As a consequence, cells grown at low temperature exhibit a resistance to photoinhibition comparable to that of cells grown at high temperature and comparable excitation pressure. We propose that the mechanism for this increased resistance to photoinhibition involves a decreased capacity to absorb incident radiation as a consequence of reduced Chl per cell coupled with lower levels of LHCII as well as increased capacity for nonradiative dissipation of excess light through Z.

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