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Photoinhibition and repair in *Dunaliella salina* acclimated to different growth irradiances

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Abstract. The light-dependent rate of photosystem-II (PSII) damage and repair was measured in photoautotrophic cultures of *Dunaliella salina* Teod. grown at different irradiances in the range 50–3000 μmol photons·m^{-2}·s^{-1}. Rates of cell growth increased in the range of 50–800 μmol photons·m^{-2}·s^{-1}, remained constant at a maximum in the range of 800–1,500 μmol photons·m^{-2}·s^{-1}, and declined due to photoinhibition in the range of 1500–3000 μmol photons·m^{-2}·s^{-1}. Western blot analyses, upon addition of lincomycin to the cultures, revealed first-order kinetics for the loss of the PSII reaction-center protein (D1) from the 32-kDa position, occurring as a result of photodamage. The rate constant of this 32-kDa protein loss was a linear function of cell growth irradiance. In the presence of lincomycin, loss of the other PSII reaction-center protein (D2) from the 34-kDa position was also observed, occurring with kinetics similar to those of the 32-kDa form of D1. Increasing rates of photodamage as a function of irradiance were accompanied by an increase in the steady-state level of a higher-molecular-weight protein complex (≈ 160-kDa) that cross-reacted with D1 antibodies. The steady-state level of the 160-kDa complex in thylakoids was also a linear function of cell growth irradiance. These observations suggest that photodamage to D1 converts stoichiometric amounts of D1 and D2 (i.e., the D1/D2 heterodimer) into a ≈160-kDa complex. This complex may help to stabilize the reaction-center proteins until degradation and replacement of D1 can occur. The results indicated an intrinsic half-time of about 60 min for the repair of individual PSII units, supporting the idea that degradation of D1 after photodamage is the rate-limiting step in the PSII repair process.

Key words: D1 protein – *Dunaliella* – Green algae – Photodamage – Photoinhibition – Photosystem II

Introduction

Light intensities exceeding those required to saturate photosynthesis cause a selective inactivation of the water-oxidizing photosystem II (PSII) in chloroplasts. This phenomenon brings about inhibition of photosynthesis, known as photoinhibition, manifested as slower rates of electron transport, lower quantum yield of photosynthesis and diminished plant growth (Kok 1956). A variety of adverse environmental conditions, such as low nutrient availability, suboptimal temperature and limited water supply accentuate photoinhibition and tend to lower the light intensity required for its onset (Powles 1984). Photoinhibition in the function of PSII occurs because of an irreversible photo-oxidative damage to a functional component in the 32-kDa (D1) reaction-center protein (Cleland et al. 1986; Barber and Andersson 1992; Prasil et al. 1992; Aro et al. 1993). Chloroplasts can recover from such rreversible photodamage through selective degradation of D1, followed by integration of a de-novo-synthesized D1 in the PSII complex (Mattoo et al. 1984; Mattoo and Edelman 1987; Adir et al. 1990).

Although at slower rates, the photodamage and replacement of D1 also occurs at subsaturating intensities of illumination in the absence of photoinhibition. Under such conditions, the rate of photodamage does not exceed the rate of repair (Greer et al. 1986) and, therefore, no adverse effect of irradiance on photosynthesis is manifested. On the other hand, under either transient or steady-state irradiance stress, the rate of photodamage might exceed the chloroplast capacity for D1 degradation and replacement, resulting in the accumulation of photochemically inactive PSII centers in the chloroplast thylakoids (Smith et al. 1990; Aro et al. 1993). The overall phenomenon of photodamage, D1 turnover and repair is now referred to as the PSII damage and repair cycle (Adir et al. 1990; Guenther and Melis 1990).

Abbreviations: Chl = chlorophyll; PSI = photosystem I; PSII = photosystem II; D1 = the 32-kDa reaction-center protein of PSII, encoded by the chloroplast *psbA* gene; D2 = the 34-kDa reaction-center protein of PSII, encoded by the chloroplast *psbD* gene; Q_A = primary electron-accepting plastoquinone of PSII

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It is encountered in all oxygen-evolving photosynthetic organisms.

Long-term exposure of plants, algae or cyanobacteria to photoinhibitory irradiance brings about structural and functional adjustments in their photosynthetic apparatus which constitute a thylakoid membrane acclimation to the adverse condition (Anderson and Osmond 1987; Long et al. 1994). Research from this laboratory has shown that the unicellular green alga *Dunaliella salina*, when exposed to long-term photoinhibition conditions, responds by decreasing the antenna size of PSI and PSII, by lowering the absolute amount of PSI in the chloroplast thylakoids, while maintaining relatively constant amounts of PSII. Under these conditions, however, a significant fraction of PSII was reported to occur as photochemically inactive centers (Smith et al. 1990; Vasilikiotis and Melis 1994). These photodamaged PSII centers contained D1 and D2 and appeared in SDS-PAGE as distinct 160-kDa complexes (Kim et al. 1993, Melis and Nemson 1995).

Initial investigations on the irradiance response of *D. salina* focused on two extreme illumination conditions, i.e., low irradiance (100 μmol photons·m^{-2}·s^{-1}) and high irradiance (2200 μmol photons·m^{-2}·s^{-1}). In the present work, we measured the rate of photodamage of the D1 protein in cells grown for several generations under different irradiances in the range 100–2500 μmol photons·m^{-2}·s^{-1}. Our results show that the rate of photodamage to PSII is a linear function of growth irradiance. At high irradiances, 160-kDa complexes, containing the D1 and D2 reaction-center proteins, accumulate in the thylakoid membrane. The results suggested that, under conditions that accelerate damage to PSII, chloroplasts are unable to rapidly process photodamaged D1 and thus are unable to efficiently restore the function of PSII.

Materials and methods

Algal culture conditions. Dunaliella salina Teod. cells were grown photoautotrophically in artificial hypersaline medium similar to that of Pick et al. (1986). Carbon was supplied as $NaHCO_3$ in the growth medium at an initial concentration of 25 mM. Cultures were grown in flat bottles (3 cm optical path length), under uniform and continuous illumination. Irradiance in the range 50–3000 μmol photons·m^{-2}·s^{-1} was provided by tungsten halogen lamps. The temperature of the cultures was maintained in the range 26–32 °C by use of water-containing heat filters. In *D. salina* cultures, the chlorophyll (Chl) content per cell remains fairly constant from the time of inoculation until the onset of the stationary phase of growth (Naus and Melis 1991). Therefore, cell growth was monitored as Chl absorbance at 678 nm in intact cells. In order to minimize light scattering, A_{678} was measured using the opal glass technique of Shibata (1958). Rates of cell duplication at different growth irradiances were calculated from the initial slope of the growth curves, in which the natural log of A_{678} was plotted as a function of time. The Chl (a + b) concentration of intact cells was determined in methanol according to Holden (1976).

Incubation with chloroplast translation inhibitors. In order to block translation of the chloroplast-encoded D1 protein, an aqueous stock solution of lincomycin was added to *D. salina* cultures to give a final concentration of 1.5 mM in the growth medium. The inhibitor was added at equivalent points during the mid exponential phase of cell growth. Cultures were maintained at their growth irradiance for the duration of the experiment, unless otherwise indicated.

Isolation of thylakoid membranes and immunochemical analysis of thylakoid proteins. Cells were harvested during the mid exponential phase of growth and thylakoid membranes were prepared by sonication and differential centrifugation as described elsewhere (Kim et al. 1993). The membrane pellets were resuspended in buffer containing 500 mM Tris-HCl (pH 6.8), 20% glycerol, 7% SDS and 2 M urea. Solubilization of protein samples was carried out for 30 min at room temperature, a process designed to prevent the formation of protein aggregates during denaturation. Chlorophyll (a + b) content of the solubilized protein samples was measured in 80% acetone by the method of Arnon (1949), with equations corrected as in Melis et al. (1987). β-Mercaptoethanol was added to give a final concentration of 10%. Samples were kept on ice until used or otherwise stored at − 80 °C. In all cases the samples were brought to room temperature prior to loading for electrophoresis. Gel lanes were loaded with an equal amount of chlorophyll (1.5 or 2 nmol depending on conditions). Protein electrophoresis was carried out with the system of Laemmli (1970), with 12.5% acrylamide in the resolving gel, in the presence of 4 M urea at a constant current of 9 mA for 16 h. Gels were stained with 1% Coomassie brilliant blue R for protein visualization or transferred to nitrocellulose. Immunoblot analysis was performed as described (Kim et al. 1993), using specific polyclonal antibodies raised against either the D1 or D2 proteins. Cross-reaction was detected via a chromogenic reaction with anti Ig-G secondary antibodies conjugated with alkaline phosphatase and it was quantified by laser densitometry with a Molecular Dynamics personal densitometer.

Results

Cell growth and chlorophyll content. The rate of cell growth in *D. salina* cultures strongly depends on the level of growth irradiance. Figure 1A shows the cell duplication rate as a function of growth irradiance in self-contained

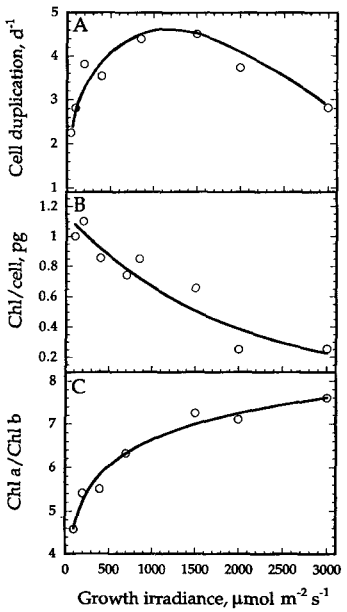

Fig. 1A–C. Growth and Chl content in *Dunaliella salina* cultures. **A** Cell duplication rate as a function of growth irradiance. The cell duplication rate (given on a per-day basis) was estimated from the initial slopes of growth curves in individual *D. salina* cultures acclimated to the indicated growth irradiance. **B** Effect of growth irradiance on the cellular Chl content in *D. salina*. **C** Effect of growth irradiance on the cellular Chl a/Chl b ratio (given as mol/mol ratio)

autotrophic cultures. At low irradiances (50–800 μmol photons·m^{-2}·s^{-1}), the rate of cell duplication increases as a function of irradiance, reflecting the light-limited growth conditions. The optimum irradiance range under the conditions of this study was between 800–1500 μmol photons·m^{-2}·s^{-1}. At higher irradiances, we observed a gradual decline in the cell duplication rate. This diminished cell growth and productivity under high light is attributed to photoinhibition of photosynthesis. The extent of this inhibition also depends on the level of irradiance (Fig. 1A).

The Chl content in *D. salina* decreased from 1 pg/cell at 100 μmol photons·m^{-2}·s^{-1} to about 0.2 pg/cell at 3000 μmol photons·m^{-2}·s^{-1}, i.e., a decline by about 80% in this range of irradiance (Fig. 1B). On the other hand, the Chl *a*/Chl *b* ratio of the cells increased from about 4.5 to about 8 as a function of growth irradiance (Fig. 1C). Taken together, these changes suggest a decrease in the Chl antenna size of the photosystems under high light, which is consistent with earlier findings from different laboratories (Sukenik et al. 1988; Smith et al. 1990).

The rate of D1 photodamage under in-vivo conditions. To investigate the effect of growth irradiance on the rate of D1 photodamage, we performed Western blot analyses of *D. salina* thylakoid proteins in the presence of the chloroplast translation inhibitor lincomycin. The inhibitor was added in the cultures during the mid exponential phase of cell growth and the cells were kept under the same irradiance at which they had been grown. Cell aliquots were harvested at different times after addition of lincomycin, processed to isolate thylakoid membranes and subjected to electrophoresis and immunoblotting. Control samples were treated in the same manner but without the addition of lincomycin. Figure 2 shows a typical experiment with *D. salina* grown at 400 μmol photons·m^{-2}·s^{-1}. The control experiment (no lincomycin added) showed a constant

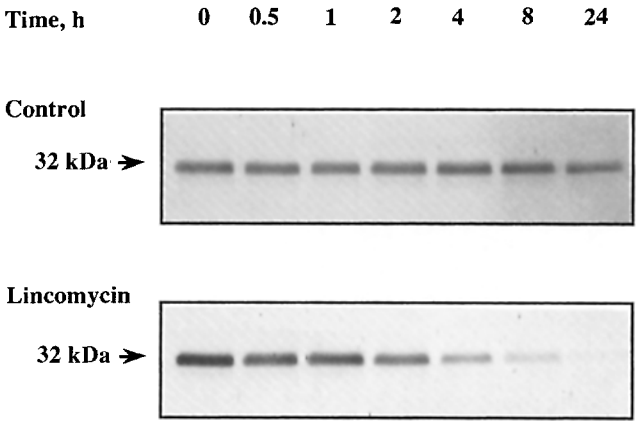

Fig. 2. Western blot analysis of *D. salina* thylakoids, probed with specific polyclonal antibodies against the D1 reaction-center protein of PSII. Cells were grown at 400 μmol photons·m^{-2}·s^{-1} to the mid exponential phase and lincomycin was added to an aliquot of the culture at a final concentration of 1.5 mM. Samples were collected as a function of time in the light after the addition of lincomycin. Upper panel (*control*): cells incubated in the absence of lincomycin. Lower panel (*lincomycin*): cells incubated in the presence of lincomycin

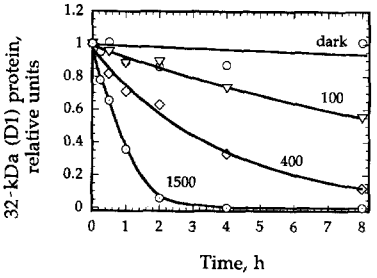

Fig. 3. Time course of D1 photodamage in *D. salina* grown at different irradiances. The amount of the 32-kDa form of D1 as a function of time after addition of lincomycin was measured by densitometric scanning of Western blots similar to those of Fig. 2. Results are expressed as a fraction of the amount of the 32-kDa protein present at zero time. Growth irradiances (in μmol photons·m^{-2}·s^{-1}) are indicated on the graph. *Dark*: cells were grown at 700 μmol photons·m^{-2}·s^{-1} and transferred to darkness immediately after addition of lincomycin

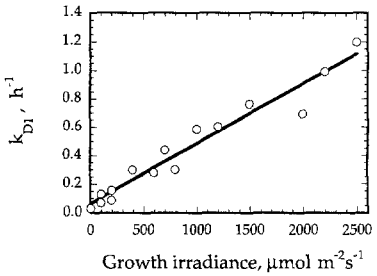

Fig. 4. Irradiance dependence of the rate constant for D1 photodamage. The first-order rate constant k_{D1} of D1 photodamage was calculated from the half-time of D1 decay traces as those shown in Fig. 3 according to the equation $k_{D1} = (\ln 2)/(t_{1/2})$

steady-state level of the D1 protein throughout the 24-h period. Upon inhibition of protein biosynthesis (Fig. 2, lincomycin), the amount of the 32-kDa protein gradually declined as a function of time in the light. This type of Western blot analysis was repeated with cells grown at different irradiances, and the resulting immunoblots were quantitated by laser densitometry. Figure 3 shows examples of such quantitations with cells grown at 100, 400 and 1500 μmol photons·m^{-2}·s^{-1}. It was observed that the decay of D1 as a 32-kDa protein band occurred with first-order kinetics. The half-time of this phenomenon was an inverse function of the cell growth irradiance. The 32-kDa form of D1 was stable when cells were incubated in the dark (Fig. 3).

Previous work from this laboratory (Kim et al. 1993) showed a direct correlation between the level of the 32-kDa form of the D1 protein and the level of PSII activity, measured spectrophotometrically at 320 nm (semiquinone anion formation). On the basis of this established relationship, the results in Fig. 3 provide a direct measure of the rate at which PSII is photodamaged under the different levels of growth irradiance. We calculated the rate constants of PSII photodamage from the half-times of traces similar to those shown in Fig. 3. The rate constant of PSII photodamage was a linear function of irradiance (Fig. 4). At a growth irradiance of 50 μmol photons·m^{-2}·s^{-1}, the

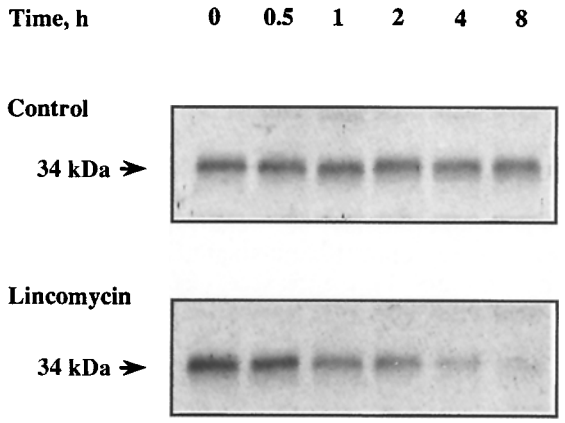

Fig. 5. Western blot analysis of *D. salina* thylakoids, probed with specific polyclonal antibodies against the D2 reaction-center protein of PSII. Cells were grown at 400 μmol photons·m^{-2}·s^{-1} to the mid exponential phase and lincomycin was added to an aliquot of the culture at a final concentration of 1.5 mM. Samples were collected as a function of time in the light after the addition of lincomycin. Upper panel (*control*): cells incubated in the absence of lincomycin. Lower panel (*lincomycin*): cells incubated in the presence of lincomycin

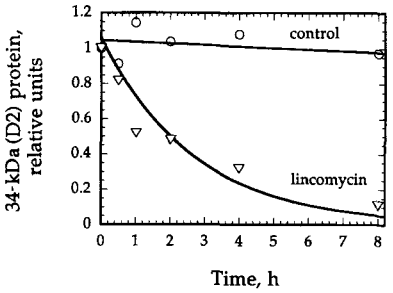

Fig. 6. Densitometric quantitation of the Western blots shown in Fig. 5. Results are expressed as a fraction of the amount of the 34-kDa protein present at zero time

rate constant for photodamage was about 0.1·h^{-1} (half-time of ≈ 7 h) whereas at 2500 μmol photons·m^{-2}·s^{-1} the rate constant for photodamage was about 1.2·h^{-1} (half-time of ≈ 35 min).

Conformational changes in PSII that follow the D1 photodamage in D. salina. Earlier work from this laboratory revealed the steady-state presence of a 160-kDa complex in the thylakoid membrane of chronically photoinhibited *D. salina*. The 160-kDa complex contained a photodamaged but as yet undegraded D1 protein (Kim et al. 1993) as well as the D2 protein of the PSII reaction center (Melis and Nemson 1995). It was postulated that the formation of such a 160-kDa complex might reflect PSII conformational changes that occur as a direct consequence of photodamage and the ensuing partial disassembly of PSII. If formation of the 160-kDa complex underlines changes in the conformation of the PSII reaction center, then one should observe a time- and irradiance-dependent loss of D2 as a distinct 34-kDa band, occurring in parallel with the loss of the 32-kDa form of D1 and corresponding with an increase in the amount of the 160-kDa complex in thylakoids. Figure 5 shows a Western blot analysis with polyclonal antibodies raised against the D2 protein. In the control samples (absence of lincomycin), there is a constant amount of the 34-kDa protein as a function of time during growth (Fig. 5, control). In the presence of lincomycin (Fig. 5, lincomycin), there is a time-dependent loss of the 34-kDa protein. Figure 6 presents a plot of densitometric scans of these results, showing that loss of D2 from the 34-kDa position occurs with first-order kinetics. At growth irradiances greater than 50 μmol photons·m^{-2}·s^{-1}, the rate constant for the loss of the 34-kDa form of D2, following addition of lincomycin to the cultures, was also a linear function of *D. salina* growth intensity (Fig. 7), occurring with a quantum yield (slope) identical to that of D1 (Fig. 4). This analysis suggests that, upon photoinhibition, stoichiometric amounts of D1 and D2 are lost from their respective positions as 32- and 34-kDa protein bands.

The results of Fig. 7 further show a biphasic dependence of k_{D2} on growth irradiance. Loss of D2 in the dark was negligible and consistent with the dark stability of D1 (Fig. 4). However, two distinct phases of the rate for the loss of D2 were evident in the 0–50 and 50–2500 μmol photons·m^{-2}·s^{-1} range (Fig. 7). The origin of this biphasic response to growth irradiance is not understood. It may be a consequence of the presence of lincomycin in the chloroplasts and/or it may relate to the high apparent quantum yield of photoinactivation observed at very low-light intensities (Keren et al. 1995; Park et al. 1995a).

Under our growth conditions, only the 32-kDa (D1) protein of PSII is subject to a frequent light-dependent photodamage and turnover (Vasilikiotis and Melis 1994). The concomitant loss of the 32-kDa (D1) and 34-kDa (D2) proteins upon addition of lincomycin can be explained by a conversion of photodamaged PSII reaction centers to a 160-kDa complex. If this were the case under physiological conditions, then one would expect thylakoid membranes of *D. salina* to contain 160-kDa complexes, the steady-state amount of which would depend on the cell growth irradiance. Figure 8 shows a Western blot analysis of thylakoid membrane proteins from *D. salina* cells grown under different irradiances. On the basis of equal Chl loading, it is evident that the steady-state amount of the 160-kDa complex increases as a function of growth

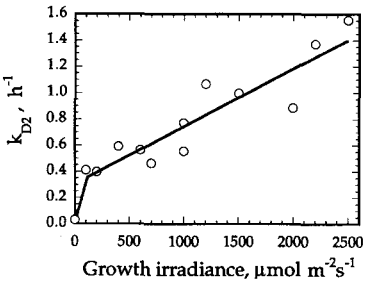

Fig. 7. Irradiance dependence of the rate constant for the 34-kDa (D2) protein loss. The first-order rate constant k_{D2} for the loss of D2 from the 34-kDa region was calculated from the half-time of D2 decay traces as those shown in Fig. 6 according to the equation $k_{D2} = (\ln 2)/(t_{1/2})$

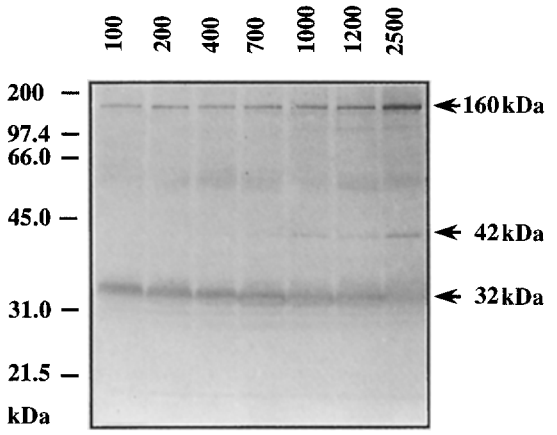

Fig. 8. Western blot analysis of *D. salina* thylakoid membrane proteins from cells grown at several different irradiances in the absence of inhibitors. Cell aliquots were harvested at the mid exponential phase of growth, thylakoids were isolated and subjected to SDS-PAGE and immunoblotting with polyclonal antibodies raised against the D1 reaction-center protein. Note the antibody cross-reaction with the 32-kDa form of D1, the 160-kDa complex and the minor cross-reaction with a 42-kDa adduct containing D1 and cytochrome *b*-559 (Barbato et al. 1992; Mori et al. 1995). Also note the increasing 160-kDa/32-kDa ratio as a function of growth irradiance

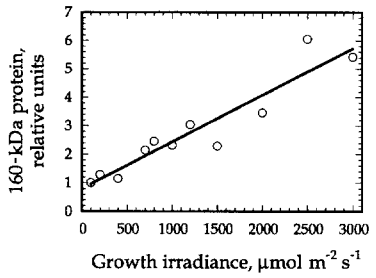

Fig. 9. Quantitation of the steady-state relative amount of the 160-kDa complex in cells grown under different irradiances. The intensity of the cross-reaction for the sample grown at 100 µmol photons·m^{-2}·s^{-1} was normalized to unity. Note the linear increase in the steady-state amount of the 160-kDa complex as a function of growth irradiance

irradiance. Figure 9 plots densitometric scans of the 160-kDa complex, revealing an approximately linear dependence of the steady-state amount of the 160-kDa complex on growth irradiance.

Discussion

Earlier work from this lab provided evidence that, under chronic photoinhibitory irradiance, thylakoid membranes in *D. salina* accumulate a significant amount of photodamaged PSII reaction centers (Kim et al. 1993; Vasilikiotis and Melis 1994). In this investigation, we report on the dynamics of this phenomenon and provide information on specific PSII conformational events that follow the D1 photodamage, examined in cells grown under a broad range of different light intensities.

The response of cell growth to the level of irradiance displays three distinct phases (light-limited, light-saturated and photoinhibition; Fig. 1A). In the light-limited range of 50–800 µmol photons·m^{-2}·s^{-1}, cell duplication rates increased with the level of irradiance, suggesting a capacity of photosynthesis sufficient to utilize irradiances up to 800 µmol photons·m^{-2}·s^{-1}. In the range of 800–1500 µmol photons·m^{-2}·s^{-1}, chloroplasts in *D. salina* have reached an upper limit in their ability to respond to further increases in the level of irradiance (light-saturated phase). For irradiances greater than 1500 µmol photons·m^{-2}·s^{-1}, the rate of cell duplication gradually declined as a result of loss in photosynthetic capacity (photoinhibition phase). The decline in the rate of cell growth, observed under photoinhibitory levels of irradiance, is attributed to the accumulation of significant amounts of photodamaged PSII centers in the thylakoid membrane (Figs. 8, 9) and to the ensuing loss of photosynthetic capacity. It should be noted that, in order to minimize the number of experimental variables, our measurements were conducted upon cell growth under continuous illumination. Clearly, diurnal oscillations in light intensity will introduce a diurnal variation in these results.

Under our growth conditions, the rate of photodamage to D1 was a linear function of irradiance (Fig. 4). Rates of photodamage between 0.05–0.7·h^{-1}, observed in the 50–1500 µmol photons·m^{-2}·s^{-1} region (Fig. 4), apparently do not exert an adverse effect on the rate of photosynthesis and cell growth (Fig. 1A). However, higher rates of photodamage, between 0.7–1.2·h^{-1}, observed in the 1500–2500 µmol photons·m^{-2}·s^{-1} region (Fig. 4), bring about a significant decline in the rate of cell growth (Fig. 1A). In principle, photoinhibition will be manifested when the rate of photodamage is approaching the rate of repair. The onset of photoinhibition in Fig. 1A appears at $\approx$ 1500 µmol photons·m^{-2}·s^{-1}; therefore, *as a first approximation*, we consider the rate of PSII repair to be about the same as the rate of photodamage at this growth irradiance. On the basis of this consideration, we estimated that the process of PSII repair in *D. salina* occurs with a rate constant of $\approx 0.7 \cdot h^{-1}$, or with a half-time of about 60 min. This estimate is comparable to the half-time for the repair of individual PSII centers in *D. salina*, obtained through independent experimentation in this laboratory (Vasilikiotis and Melis 1994).

The linear dependence of the rate of photodamage on growth irradiance (Fig. 4) may suggest that the probability of photodamage is a direct function of the number of charge separation reactions that occur at PSII. According to this scenario, there is an inherent probability for PSII photodamage every time there is a charge separation between P680 and pheophytin. At higher light intensities, the frequency of charge separation is increased, thereby increasing the rate of photodamage. This hypothesis is consistent with recent findings by Park et al. (1995b) who observed a reciprocity of irradiance and duration of illumination for PSII function, suggesting that PSII photodamage depends on the total number of photons absorbed and not so much on the rate of photon absorption.

The probability for photodamage could be quite different in the two redox states of the PSII reaction center

(Q_A in the oxidized or reduced form during steady-state electron flow). For example, there may be a low inherent probability for photodamage when forward electron-transport takes place, i.e., when Q_A is oxidized in steady-state electron transport, and a significantly higher probability when forward electron-transport is blocked, i.e., when Q_A remains in the reduced state. This assertion is consistent with results showing that environmental conditions that block photosynthesis, i.e., low-temperature or lack of CO_2, accentuate photoinhibition at a fixed light intensity (Powles 1984). Also consistent with this view is the proposal by Maxwell et al. (1994, 1995) that the rate of photodamage depends primarily on PSII "excitation pressure", defined by the amount of light that is absorbed by PSII but cannot be utilized photochemically because of the redox poise of plastoquinone and of the primary quinone acceptor Q_A. According to this concept, the linear increase in the rate of photodamage as a function of growth irradiance (Fig. 4) implies a linear increase in the fraction of Q_A that remains reduced during steady-state electron transport. Such a linear dependence in the steady-state amount of reduced Q_A as a function of incident light intensity was demonstrated in work with suspended protoplasts of barley by Krömer et al. (1993).

The loss of D2 as a 34-kDa protein in Western blots, observed upon photoinhibition of PSII and occurring with kinetics similar to those of D1 (Figs. 5, 6) does not necessarily indicate photodamage and turnover of the D2 protein. Rather, it underlines a photodamage-dependent conversion of the D1/D2 heterodimer into a 160-kDa complex in which D1 is degraded and replaced whereas D2 is recycled into a newly functional PSII center (D2 is not turning over in *Dunaliella*; Vasilikiotis and Melis 1994). This interpretation is consistent with the identification of D2 as a constituent protein of the 160-kDa complex (Melis and Nemson 1995) and with the observation that the rate of D2 loss is a linear function of growth irradiance (Fig. 7), occurring with a quantum yield (slope) identical to that of D1 (Fig. 4).

The steady-state level of photodamage can be measured from the relative amount of 160-kDa complexes that occur in *D. salina* thylakoids (Fig. 9). Under low and moderate growth irradiances, accumulation of small amounts of the 160-kDa complex will not entail photoinhibition because the photodamage of a small fraction of PSII centers will be compensated from the reserve pool of $PSII_\beta$ centers (about 25% of the total PSII) in the chloroplast thylakoids (Neale and Melis 1990; 1991). Under high irradiances, the reserve pool of $PSII_\beta$ centers will be depleted and photoinhibition will then be manifested. Indeed, growth of *D. salina* at 2200 μmol photons·m^{-2}·s^{-1} entails the accumulation of $\approx 80\%$ of PSII reaction centers in a photochemically inactive state (Vasilikiotis and Melis 1994) and the manifestation of photoinhibition (Fig. 1A).

The 160-kDa complex may serve as a repair cycle intermediate, and/or it may represent a structural stage that follows the disassembly of the PSII complex. This configuration may be attained prior to the degradation and removal of the photodamaged D1. This hypothesis is consistent with the observation that photodamage will occur at all irradiances (Fig. 4) and also consistent with the fact that 160-kDa complexes are detected at irradiances lower than those required for the onset of photoinhibition (Fig. 8). Thus, the formation of the 160-kDa complex is an interesting observation and merits further investigation. Clearly, more work is needed to dissect the structural configuration and functional properties of the 160-kDa complex.

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