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# Photosystem II reaction center damage and repair cycle: Chloroplast acclimation strategy to irradiance stress

(thylakoid membrane/D1 protein/*Dunaliella salina*/pulse labeling)

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ABSTRACT **A daily occurrence in the life of a plant is the function of a photosystem II (PSII) damage and repair cycle in chloroplasts. This unique phenomenon involves the frequent turnover of D1, the 32-kDa reaction-center protein of PSII (chloroplast *psbA* gene product). In the model organism *Dunaliella salina* (a green alga), growth under low light (100 μmol of photons per $m^2$ per sec) entails damage, degradation, and replacement of D1 every 7 hr. Growth under irradiance stress (2200 μmol of photons per $m^2$ per sec) entails damage to D1 every 20 min. The rate of *de novo* D1 biosynthesis under conditions of both low light and irradiance stress was found to be fairly constant on a per chloroplast or cell basis. The response of *D. salina* to the enhanced rate of damage entails an accumulation of photodamaged centers (80% of all PSII) and the formation of thylakoid membranes containing a smaller quantity of photosystem I (PSI) centers (about 10% of that in cells grown under low light). These changes contribute to a shift in the PSII/PSI ratio from 1.4:1 under low-light conditions to 15:1 under irradiance stress. The accumulation of photodamaged PSII under irradiance stress reflects a chloroplast inability to match the rate of D1 degradation or turnover with the rate of damage for individual PSII complexes. The altered thylakoid membrane organization ensures that a small fraction of PSII centers remains functional under irradiance stress and sustains electron flow from $H_2O$ to ferredoxin with rates sufficient for chloroplast photosynthesis and cell growth.**

The photosynthetic performance of a plant can be severely inhibited following exposure to light intensities in excess of those required to saturate photosynthesis (1). The threshold intensity for the onset of this photoinhibition can be very low, as in shade-adapted species and in plants where other environmental stresses (e.g., chilling, drought, lack of $CO_2$, heat stress) have a synergistic effect with irradiance stress (2). Photoinhibition adversely affects the function of photosystem II (PSII) in chloroplasts, and it is manifest as lowered rates of electron transport and oxygen evolution, resulting in a lower quantum yield and a lower light-saturated rate of photosynthesis (3). It is generally accepted that photoinhibition entails damage to a functional component in D1, the 32-kDa reaction-center protein of PSII encoded by the chloroplast *psbA* gene (4, 5).

It has been proposed that such damage occurs even under physiological conditions and that it is the underlying reason for the frequent turnover of D1 (6, 7). This protein contains all of the functional cofactors in PSII and functions in the oxidation of water and reduction of plastoquinone in chloroplasts (8). It accounts for <1% of the total thylakoid membrane protein content, yet the rate of its synthesis is comparable to that of the abundant large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) in the chloroplast (9–12).

The high turnover rate of D1 underlines frequent damage and the need for replacement of this protein in the PSII complex. Thus, PSII in chloroplasts undergoes a frequent damage and repair cycle (13, 14). Under optimal growth conditions, the rate of photodamage does not exceed the rate of repair, therefore, no adverse effect on photosynthesis is manifest. Under transient or steady-state irradiance stress, however, the rate of photodamage might exceed the rate of repair in the chloroplast, resulting in accumulation of damaged PSII centers in the thylakoid membrane (2, 6, 15).

Recent work from this laboratory has shown that in the model organism *Dunaliella salina* (a green alga), chronic irradiance stress induces structural and functional modifications in the organization of thylakoid membranes and in the PS composition (16, 17). These modifications are significant because they permit cells to resume photosynthesis and growth under adverse irradiance. They include a much higher PSII/PSI ratio in thylakoids of cells grown in high light (HL cells) compared with that in thylakoids of cells grown in low light (LL cells). However, most of the PSII units in HL-grown thylakoids contain a damaged form of the D1 protein and are photochemically inactive (16). In this article, we show that the rate of D1 biosynthesis is fairly constant when measured on a per chloroplast basis under conditions of LL or irradiance stress, and it is only slightly enhanced when measured on the basis of the total PSII ($PSII_t$) content in chloroplasts. We further show that irradiance stress induces a significant lowering of the amount of PSI in chloroplasts and the concomitant accumulation of damaged PSII centers in thylakoids. We propose that the successful cell acclimation strategy to irradiance stress involves the formation of large pools of photoinactivated PSII centers that serve as sites for the degradation and replacement of D1.

## MATERIALS AND METHODS

*D. salina* cultures were grown in an artificial hypersaline medium similar to that of Pick *et al.* (18) as described (19). Thylakoid membrane isolation, chlorophyll (Chl) and PSII activity measurements, and the SDS/PAGE and immunoblot analyses have been described (19, 20).

To measure the rate of synthesis of thylakoid membrane proteins, *D. salina* cultures were pulse-labeled with [$^{35}$S]sulfate. Late logarithmic-phase cultures were harvested by centrifugation at 1000 × *g* for 2 min at 4°C and resuspended in a growth medium without sulfate for 4 hr to remove stored sulfur. Cultures were subsequently illuminated in the pres-

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Abbreviations: Chl, chlorophyll; PS, photosystem; D1, the 32-kDa reaction-center protein of PSII encoded by the chloroplast *psbA* gene; HL, high light; LL, low light; HL and LL cells, cells grown in HL and LL, respectively; $Q_A$, primary electron-accepting plastoquinone of PSII; PSII*, functional PSII; $PSII_t$, total PSII (PSII* and photoinactivated centers); Cyt $b_{559}$, cytochrome $b_{559}$; $t_{1/2}$, half-time.

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ence of 6 μCi (222 kBq) per ml of [$^{35}$S]sulfate. Aliquots were harvested at 15-min intervals, and cells were precipitated by centrifugation at 3000 × *g* for 3 min at 4°C, resuspended in sonication buffer, and stored on ice until all samples were collected. Cells were then disrupted by sonication, thylakoid membranes were isolated, and proteins were resolved on SDS/PAGE (19). Gels were stained with Coomassie brilliant blue R and dried onto filter paper. Dry gels were exposed to either x-ray film for autoradiography or to Molecular Dynamics storage phosphor screens. The x-ray films were scanned, and the amount of label incorporated into the D1 protein was quantitated by using an LKB-Pharmacia XL laser densitometer. The phosphor screens were scanned and quantitated with the Molecular Dynamics PhosphorImager.

Slot immunoblot analysis for the quantitation of reaction-center polypeptides was performed with a Hoefer Slot Blot model PR648 apparatus and with specific polyclonal antibodies against the PSII reaction-center proteins—i.e., the D1 (*psbA* gene) protein and the 9-kDa subunit of the cytochrome $b_{559}$ (Cyt-$b_{559}$; *psbE* gene) protein of spinach.

## RESULTS

**Cell Growth, Chlorophyll, and Photosystem Content in *D. salina*.** Rates of *D. salina* growth under the LL (100 μmol of photons per m$^2$ per sec) and HL (2200 μmol of photons per m$^2$ per sec) culture conditions were estimated from the slope, *S*, at the logarithmically linear phase of the respective growth curves (19, 20). Our measurements indicated a $S_{LL}$ = 2.0 days$^{-1}$ and a $S_{HL}$ = 1.5 days$^{-1}$, suggesting a slightly faster cell growth under LL than HL conditions. It is known that *D. salina* grown under optimal light intensities is capable of higher rates of growth at the natural log–linear phase, approaching about 5 days$^{-1}$ at an incident intensity of 500 μmol of photons per m$^2$ per sec (16). Thus, under our LL culture conditions, a $S_{LL}$ = 2.0 days$^{-1}$ suggests growth limited by the intensity of illumination. Under our irradiance-stress conditions, a $S_{HL}$ = 1.5 days$^{-1}$ underlines a chronic photoinhibition condition that impedes photosynthesis and cell growth.

Cultures under conditions of LL and irradiance stress reached about the same density of 2 × 10$^6$ cells per ml. However, they differed substantially in the Chl/cell ratio. The LL cultures have a Chl/cell ratio of 1.00 × 10$^9$, whereas the HL cultures have a ratio of 0.13 × 10$^9$. Additionally, there is a significant difference in the Chl a/Chl b ratio, from a 5:1 ratio in LL cells to a 18:1 ratio in HL cells (Table 1). A higher Chl a/Chl b ratio and a lower Chl/cell ratio suggest a smaller photosynthetic unit size under irradiance stress (16, 19, 21).

Acclimation to irradiance stress involves further changes in the PS content of thylakoid membranes and in the PSII/PSI stoichiometry. Table 1 presents a summary of the photochemical apparatus characteristics in *D. salina*. The ratio of Chl/PSI is 650:1 in LL cells and 1040:1 in HL cells. The ratio of Chl to functional PSII, PSII*, is 440:1 in LL cells and 340:1 in HL cells. The ratio of functional photosystems (PSII*/PSI) is 1.4:1 in LL cells and 3.1:1 in HL cells (Table 1). A PSII*/PSI = 3.1:1 in HL cells is a response of the plant to the much smaller Chl antenna size for PSII under HL conditions (16, 21) and ensures a balanced electron flow between the two photosystems in the thylakoid membrane (22).

Earlier work from this laboratory (16, 19) has shown that under irradiance stress, thylakoids accumulate a significant amount of photoinactivated PSII centers that contain a damaged D1. These photoinactivated centers account for the majority of PSII and further add to the stoichiometric imbalance between PSII and PSI in HL-grown thylakoids (16, 17). To assess the significance of this peculiar structural/functional organization of thylakoids under chronic irradiance stress and to further explore the operation of the PSII repair cycle in chloroplasts, we used immunoblot analysis and [$^{35}$S]sulfate labeling to measure, respectively, the PSII concentration and the rate of D1 biosynthesis in chloroplasts under LL and HL conditions.

**Immunoblot Quantitation of the Total Number of PSII Reaction Centers.** Photoinactivated centers cannot perform a charge separation and can be detected only with immunochemical approaches (16, 19). To compare the PSII$_t$ concentration (PSII* and photoinactivated PSII) in thylakoid membranes from LL and HL cells, we measured the total amount of PSII polypeptides that cross-react with specific polyclonal antibodies. Thylakoid membrane samples from LL cells and HL cells were loaded on a slot-blot apparatus and probed with antibodies raised against either D1 or the Cyt $b_{559}$ 9-kDa apoprotein. Fig. 1 shows the results from a laser densitometric analysis of the cross-reaction in slot immunoblots, loaded with a series of concentrations of Chl from LL- and HL-grown samples. From the slopes of the fitted lines in Fig. 1, it is evident that, on the basis of comparable Chl content, HL-grown thylakoids contain about 5.5-times more D1 (Fig. 1 *Upper*) and 6.7-times more Cyt $b_{559}$ (Fig. 1 *Lower*) than LL-grown thylakoids. Assuming that LL-grown thylakoids contain a negligibly small amount of photoinactivated PSII centers (23), we estimated that the Chl/PSII$_t$ ratio is 70:1 in HL-grown thylakoids (Table 2).

The above analysis suggested that irradiance stress induced significant changes in the organization and function of thylakoids. Table 2 shows that the PSII*/PSII$_t$ ratio is 1:1 in LL cells and 0.21.1 in HL cells, suggesting that about 80% of the PSII$_t$ in HL cells consists of photodamaged centers.

The PSII$_t$/PSI ratio in HL cells is 15/1, compared with a 1.4/1 ratio for LL cells (Table 2). The lopsided PSII$_t$/PSI ratio in HL cells could not be explained as being due to an increase in the PSII content of thylakoids. Examination of the PSI and PSII contents on a per chloroplast or cell basis revealed that HL cells retain about 64% of the PSII found in LL cells; however, they contain a mere 6–7% of the amount of PSI found in LL cells (Table 2). Thus, the response of *D. salina* to irradiance stress includes a lower absolute amount of PSI complexes in thylakoids and, at the same time, the formation of a sizable pool of photoinactivated PSII centers that contain a damaged D1 protein. The latter could be a specific response of the plant to the more frequent PSII reaction-center damage under irradiance stress, and it may be significant for the repair of damaged centers.

**Rate of Synthesis of D1 Under LL and HL Cell Culture Conditions.** A recent study from this laboratory has shown

Table 1. Photochemical apparatus characteristics in *D. salina*

| Conditions | Chl/cell ratio × 10$^{-9}$ | mol/mol ratio | | | |
|---|---|---|---|---|---|
| | | Chl a/Chl b | Chl/PSI | Chl/PSII* | PSII*/PSI |
| LL | 1.00 | 5:1 | 650:1 | 440:1 | 1.4:1 |
| HL | 0.13 | 18:1 | 1040:1 | 340:1 | 3.1:1 |

Pigment content and photosynthetic membrane component quantitation are given for LL (100 μmol of photons per m$^2$ per sec)- and HL (2200 μmol of photons per m$^2$ per sec)-grown *D. salina*. Functional PSII (PSII*) and PSI centers were estimated from spectrophotometric measurements of $Q_A$ photoreduction (PSII*) and P700 photooxidation (PSI). Component quantitation (mol:mol ratios) and Chl/cell ratios (absolute number of Chl molecules per cell) are given on a total Chl (a + b) basis.

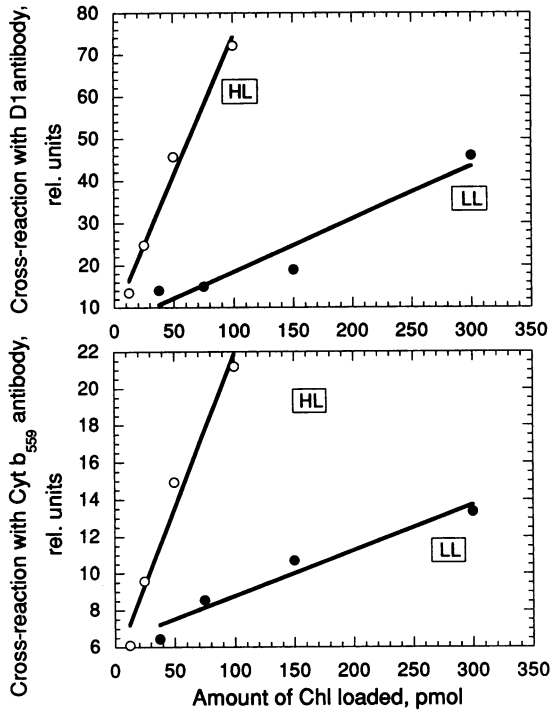


FIG. 1. Immunoblot analyses of total *D. salina* thylakoid membrane proteins; quantitation of the cross-reaction between polyclonal antibodies and PSII reaction center proteins in LL- and HL-grown *D. salina* as determined by laser densitometry. A range of Chl concentrations, from 37.5 to 300 pmol (LL) and from 12.5 to 200 pmol (HL), was loaded into the wells of a slot-blot apparatus and probed with antibodies raised against D1 (*Upper*) or the 9-kDa subunit of the Cyt $b_{559}$ (*Lower*). On the basis of Chl content, HL-grown thylakoids contained about 5.5 times more D1 and 6.7 times more Cyt $b_{559}$ than did LL-grown thylakoids. rel., Relative.

that photoinactivation (damage) to D1 occurs with a half-time ($t_{1/2}$) of 7 hr under LL growth conditions, compared with a $t_{1/2}$ of 20 min under HL conditions (19). Acclimation of *D. salina* to irradiance stress implied a correspondingly higher rate of repair as compared with that under LL conditions. The regulation of D1 biosynthesis, the acclimation strategy used by the cells, and the function of the repair cycle under irradiance stress are not understood at present. To address the question of this regulation, we investigated the rate of biosynthesis of D1 under LL and HL culture conditions and correlated the rate of D1 biosynthesis to the thylakoid content in PSI, PSII*, and $PSII_t$.

Cells were pulse-labeled with [$^{35}$S]sulfate, and thylakoid membrane proteins were isolated and resolved on SDS/PAGE (24) (Figs. 2 and 3). Lanes were loaded with 6 nmol of Chl (a+b) (LL-grown *D. salina*, Fig. 2) or with 2.1 nmol of Chl (a+b) (HL-grown *D. salina*, Fig. 3). Even though the steady-state amount of D1 is only a small fraction of the total thylakoid membrane protein, it exhibits the highest rate of biosynthesis among all thylakoid proteins as judged by the rate of incorporation of the [$^{35}$S]sulfate label into D1 for LL (Fig. 4 *Left*) and HL (Fig. 4 *Right*) cultures. Fig. 5 presents a quantitation of the [$^{35}$S]sulfate label incorporated into D1 on the basis of equal amounts of Chl in LL cells and HL cells. From the initial slope of the fitted curves, it appears that the HL cells incorporate label into the D1 protein at a rate that is about 8-fold faster than that in LL cells.

Table 3 presents a summary of the rate of D1 biosynthesis data. Of interest is the observation that the rate of D1 biosynthesis per PSI (Table 3) is about 1 order of magnitude faster in HL than LL cells. This observation suggests that, on the basis of a one-electron transport chain (PSI) in thylakoid membranes, the rate of D1 turnover is significantly faster in HL than LL chloroplasts. Of greater interest is the observation that the rate of D1 label incorporation per chloroplast or cell does not differ significantly in LL and HL cells. The rate of D1 label incorporation per PSII* was about 6 times faster in HL than LL cells; however, the rate of D1 label incorporation per $PSII_t$ in HL cells was enhanced by only about 30% over that in LL cells (Table 3). These interesting results suggest that cells maintain a constant rate of D1 biosynthesis as a function of irradiance and that the main response of *D. salina* cells to irradiance stress is structural and functional modification of thylakoid membranes, including a lowering of the concentration of PSI and the formation of a large pool of photoinactivated PSII centers in chloroplasts.

## DISCUSSION

The acclimation strategy to photoinhibitory irradiance stress in *D. salina* involves a significant lowering of the quantity of PSI in chloroplasts and accumulation of damaged PSII centers with the D1 protein still attached in thylakoid membranes. A lowering of the concentration of PSI in chloroplasts can be viewed as a response to the level of irradiance seen by the cells: at high irradiance, fewer PSI complexes are needed to carry out electron flow from the cytochrome $b_6$-$f$ complex to ferredoxin. It is important to note that the total number of PSII centers per chloroplast in *D. salina* does not change significantly as a function of irradiance, an observation contrary to that for PSI centers. Thus, because the concentration of PSI centers in HL cells is reduced to about 6.6% of that in LL cells, the ratio of $PSII_t$/PSI changes from 1.4:1 in LL cells to 15:1 in HL cells (Table 2). The majority of $PSII_t$ centers in HL cells (about 80% of the $PSII_t$) occur as photodamaged centers (Fig. 1) and do not participate in the process of electron transport (Table 2). Thus, steady-state photosynthetic electron transport from $H_2O$ to ferredoxin in HL-grown *D. salina* is sustained by only about 20% of the $PSII_t$ centers in thylakoids and by about 10% of the potential cellular PSI content.

There is precedence in the literature for the down-regulation of PSI biosynthesis–assembly in chloroplasts. Studies on the chromatic regulation of PS stoichiometry in cyanophytes and higher-plant chloroplasts have shown that the PSII/PSI ratio in thylakoids is adjusted and optimized in response to the quality of light during growth (4). Adjustments of PS stoichiometry in chloroplasts improve the quan-

Table 2. Photosystem stoichiometry in *D. salina*

| Conditions | mol/mol ratio | | | $PSII_t$, no. per cell | PSI, no. per cell |
|---|---|---|---|---|---|
| | Chl/$PSII_t$ | PSII*/$PSII_t$ | $PSII_t$/PSI | | |
| LL | 440:1 | 1:1 | 1.4:1 | $1.75 \times 10^6$ | $1.19 \times 10^6$ |
| HL | 70:1 | 0.21:1 | 15.0:1 | $1.12 \times 10^6$ | $0.08 \times 10^6$ |
| | | | | 64%† | 6.6%† |

Quantitations are given for LL (100 μmol of photons per m$^2$ per sec)- and HL (2200 μmol of photons per m$^2$ per sec)-grown *D. salina*. The relative amount of $PSII_t$ was obtained from immunoblot analysis (see Fig. 1).

†% remaining (in HL cells)

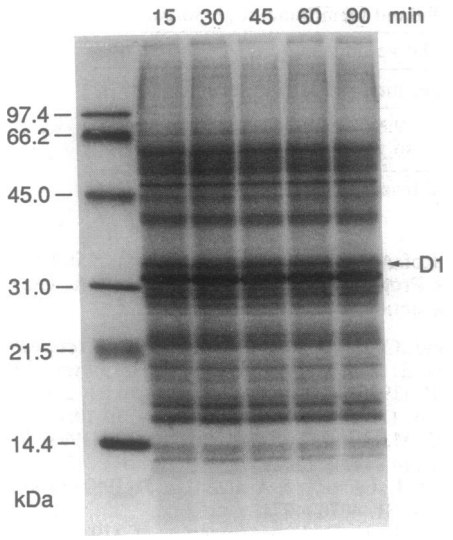


FIG. 2. Profile of Coomassie-stained thylakoid membrane proteins from LL-grown *D. salina*. Late logarithmic-phase cultures were pulse-labeled with [$^{35}$S]sulfate, and aliquots were removed at the time intervals indicated. The thylakoid membranes were isolated, and samples containing 6 nmol of Chl (a+b) were loaded on SDS/PAGE.

tum efficiency of photosynthesis (25). Experimental evidence has shown that such changes in the PSII/PSI ratio are mainly adjustments in the chloroplast concentration of PSI (26, 27). This is manifest both as variable amounts of PSI per chloroplast (26) and variable steady-state levels of *psaA* gene products (28) occurring in contrast to the constancy in PSII. Thus, there appear to be a constitutive expression of PSII and a regulated expression of PSI in oxygenic photosynthesis.

Under conditions of LL and under irradiance stress, the rate of *de novo* D1 biosynthesis per cell appears to be fairly constant. This finding is important in terms of the acclimation of chloroplasts to chronic photoinhibition conditions. Clearly, the acclimation strategy to irradiance stress in *D. salina* does not involve a substantial acceleration of the PSII repair cycle for individual PSII complexes. Assuming that a PSII damage and repair cycle can be approximated by a

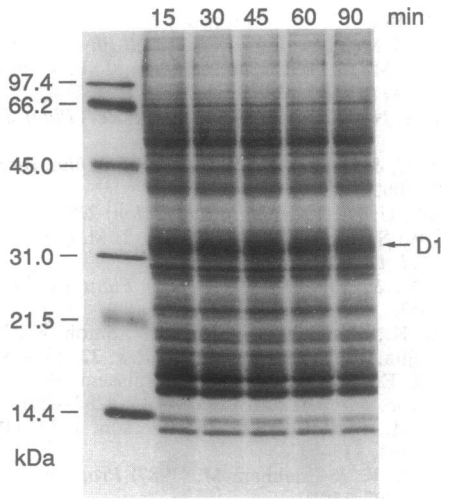


FIG. 3. Profile of Coomassie-stained thylakoid membrane proteins from HL-grown *D. salina*. Late logarithmic-phase cultures were pulse-labeled with [$^{35}$S]sulfate, and aliquots were removed at the time intervals indicated. The thylakoid membranes were isolated and samples containing 2.1 nmol of Chl (a+b) were loaded on SDS/PAGE.

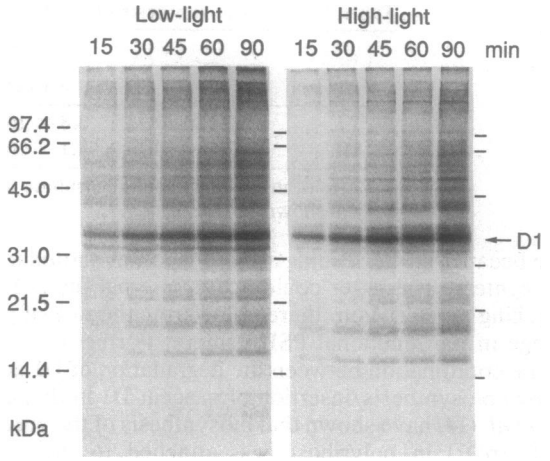


FIG. 4. PhosphorImager printout of thylakoid membrane proteins labeled with [$^{35}$S]sulfate and resolved on SDS/PAGE. LL- and HL-grown *D. salina* cultures were pulse-labeled for 15–90 min, and thylakoid membranes were isolated and loaded on SDS/PAGE [6 nmol Chl (a+b) for LL- and 2.1 nmol Chl (a+b) for HL-grown thylakoids]. Note that the D1/32-kDa polypeptide exhibited the highest rate of *de novo* synthesis among all thylakoid membrane proteins in both LL- and HL-grown *D. salina*.

first-order reversible reaction ($A \underset{r}{\overset{d}{\rightleftharpoons}} B$), we estimated a $t_{1/2}$ of about 80 min for the repair of individual PSII complexes, independent of the light intensity during growth (analysis not shown). This finding explains why no photoinactivated centers accumulate in the thylakoid membrane of LL-grown cells [$t_{1/2}$ (damage) = 7 hr; $t_{1/2}$ (repair) = 80 min], whereas a significant amount of photoinactivated PSII centers accumulate in the thylakoid membrane of HL-grown cells [$t_{1/2}$ (damage) = 20 min; $t_{1/2}$ (repair) = 80 min]. Thus, accumulation of damaged PSII centers in thylakoids under irradiance stress reflects a chloroplast inability to accelerate the rate of D1 turnover for individual PSII complexes. These results suggest that degradation of damaged D1 proteins is regulated by an as yet unknown mechanism and that degradation of damaged D1 is the rate-limiting step in the PSII damage and repair cycle (19, 29).

The reason for the constancy of the PSII content in chloroplasts and for the limitation in the rate of D1 degradation/repair for individual PSII complexes is not fully understood at present. Slow rates of D1 degradation may be seen as a regulatory mechanism for the PSII damage and repair

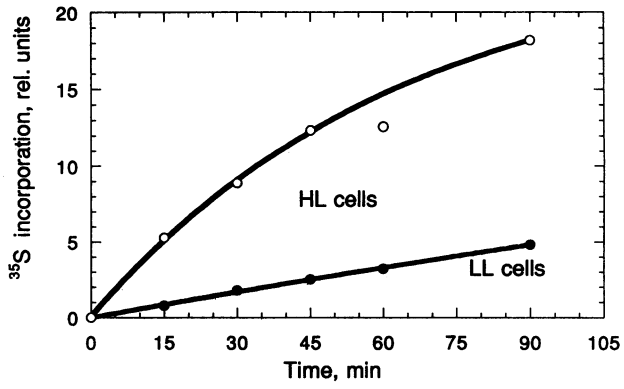


FIG. 5. Rate of incorporation of [$^{35}$S]sulfate label (relative units) into the D1/32-kDa protein in LL- and HL-grown *D. salina*. The amount of [$^{35}$S]sulfate label was calculated on the basis of equal amounts of Chl (a+b) and plotted as a function of time (duration of labeling). The rate of synthesis of D1 in HL-grown cultures was about 8 times faster than that in LL-grown cultures. The results are the average values of three separate experiments.

Table 3. Rates of synthesis of the D1/32-kDa protein in the thylakoid membrane of *D. salina*

| Conditions | $^{35}S$ incorporated into D1, relative units | | | | |
|---|---|---|---|---|---|
| | per mol of Chl | per cell | per mol of PSI | per mol of PSII* | per mol of $PSII_t$ |
| LL | $2.2 \times 10^{12}$ | $3.8 \times 10^{-3}$ | $1.5 \times 10^{15}$ | $9.8 \times 10^{14}$ | $9.8 \times 10^{14}$ |
| HL | $17.6 \times 10^{12}$ | $4.1 \times 10^{-3}$ | $17.7 \times 10^{15}$ | $59.2 \times 10^{14}$ | $12.3 \times 10^{14}$ |

Rates of incorporation of [$^{35}S$]sulfate label in D1 were estimated from the initial slopes of the fitted curves shown in Fig. 5.

cycle because of the ensuing accumulation of photodamaged PSII centers. The latter could act as internal filters for the quenching of excitation, thereby lowering the rate of photodamage in the functional PSII centers. Furthermore, there may be coordination between the degradation of D1 and the *de novo* biosynthesis/insertion of nascent D1 in thylakoids. Adir *et al.* (14) have shown that biosynthesis of the precursor of D1 (pD1) in polyribosomes, attached to the stroma-exposed thylakoids, is coupled with the integration of D1 into existing PSII centers (30). The rate of this process may be limited by the rate of degradation of damaged D1. A coupling between D1 biosynthesis and integration into PSII centers may help to stabilize the newly synthesized pD1, which otherwise would have been rapidly degraded. A similar translational control has been described for the biosynthesis of Chl-binding proteins (31).

Of interest is the consequence of chronic irradiance stress on the structural–functional status of *D. salina* thylakoids. The latter are depleted in PSI and simultaneously are enriched in photoinactivated PSII centers (Table 2). Thus, the thylakoid membrane domain occupied by photodamaged PSII centers is significantly larger than that occupied by photochemically competent PSII* and PSI centers. This consideration suggests that, under chronic irradiance stress, a significant surface area of the thylakoid membrane functions to house photoinactivated centers. It further raises the question of whether these photoinactivated centers exist in distinct thylakoid domains that may serve as a site for the D1 degradation, *de novo* biosynthesis-insertion, and reassembly processes that constitute the essence of the PSII repair. The possible occurrence of extensive PSII "repair stations" containing a large pool of photoinactivated PSII centers is consistent with previous studies from this laboratory in which accelerated damage to the D1 protein has been correlated with the appearance of a 160-kDa complex that presumably consists of a dimer of PSII reaction centers (19). These 160-kDa dimers are found only in irradiance-stressed cells under conditions of chronic photoinhibition and appear in SDS/PAGE upon solubilization of thylakoid membranes from HL cells. This peculiar configuration of PSII in thylakoids of HL cells may help to stabilize damaged PSII centers until they can undergo the process of repair.

The acclimation strategy to chronic irradiance stress by *D. salina* appears to be distinctly different from the strategy employed by cyanobacteria. Recent studies have shown that *Synechococcus* sp. PCC 7942 responds to irradiance stress by interchanging two distinct forms of the D1 protein (D1:1 and D1:2) (32, 33). The D1:1 form is expressed under low and moderate light intensities but is rapidly replaced by a second form, D1:2, under high-irradiance conditions. The D1:2 form is apparently less susceptible to photoinhibition than D1:1. Alternatively, PSII centers containing D1:2 may have a higher capacity of repair compared with PSII centers containing D1:1. It appears then that cyanobacteria respond to photoinhibition by rapidly replacing the existing D1 protein with another form that provides PSII centers either with increased resistance to photoinhibition or facilitates a more rapid recovery.

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1. Osmond, C. B. (1981) *Biochim. Biophys. Acta* **639**, 77–98.
2. Powles, S. B. (1984) *Annu. Rev. Plant. Physiol.* **35**, 15–44.
3. Kok, B. (1956) *Biochim. Biophys. Acta* **21**, 234–244.
4. Melis, A. (1991) *Biochim. Biophys. Acta* **1058**, 87–106.
5. Aro, E.-M., Virgin, I. & Andersson, B. (1993) *Biochim. Biophys. Acta* **1143**, 113–134.
6. Kyle, D. J., Ohad, I. & Arntzen, C. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4070–4074.
7. Ohad, I., Kyle, D. J. & Arntzen, C. J. (1984) *J. Cell. Biol.* **99**, 481–485.
8. Barber, J. (1989) in *Oxford Surveys of Plant Molecular and Cell Biology*, ed. Miflin, B. J. (Oxford Univ. Press, New York), Vol. 6, pp. 115–162.
9. Bottomley, W., Spencer, D. & Whitfeld, P. (1974) *Arch. Biochem. Biophys.* **164**, 106–117.
10. Eaglesham, A. & Ellis, R. (1974) *Biochim. Biophys. Acta* **335**, 396–407.
11. Edelman, M. & Reisfeld, A. (1978) in *Chloroplast Development*, eds. Akoyunoglou, G. & Argyroudi-Akoyunoglou, J. H. (Elsevier/North Holland, New York), pp. 641–652.
12. Mattoo, A., Hoffman-Falk, H., Marder, J. & Edelman, M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1380–1384.
13. Guenther, J. E. & Melis, A. (1990) *Photosynth. Res.* **23**, 105–110.
14. Adir, N., Shochat, S. & Ohad, I. (1990) *J. Biol. Chem.* **265**, 12563–12568.
15. Greer, D. H., Berry, J. A. & Björkman, O. (1986) *Planta* **168**, 253-260.
16. Smith, B. M., Morrissey, P. J., Guenther, J. E., Nemson, J. A., Harrison, M. A., Allen, J. F. & Melis, A. (1990) *Plant. Physiol. (Bethesda)* **93**, 1433–1440.
17. Melis, A. (1992) in *Regulation of Chloroplast Biogenesis*, NATO ASI Series A: Life Sciences, ed. Argyroudi-Akoyunoglou, J. H. (Plenum, New York), Vol. 226, pp. 491–498.
18. Pick, U., Karni, L. & Avron, M. (1986) *Plant Physiol.* **81**, 92–96.
19. Kim, J. H., Nemson, J. A. & Melis, A. (1993) *Plant Physiol.* **103**, 181–189.
20. Naus, J. & Melis, A. (1991) *Plant Cell Physiol.* **32**, 569–576.
21. Harrison, M. A., Melis, A. & Allen, J. F. (1992) *Biochim. Biophys. Acta* **1100**, 83–91.
22. Glazer, A. N. & Melis, A. (1987) *Annu. Rev. Plant. Physiol.* **38**, 11–45.
23. Ley, A. C. & Mauzerall, D. C. (1982) *Biochim. Biophys. Acta* **680**, 95–106.
24. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
25. Chow, W. S., Melis, A. & Anderson, J. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7502–7506.
26. Fujita, Y. & Murakami, A. (1987) *Plant Cell Physiol.* **28**, 1547–1553.
27. Aizawa, K., Shimizu, T., Hiyama, T., Satoh, K., Nakamura, Y. & Fujita, Y. (1992) *Photosynth. Res.* **32**, 131–138.
28. Glick, R. E., McCauley, S. W., Gruissem, W. & Melis, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4287–4291.
29. Aro, E.-M., McCaffery, S. & Anderson, J. M. (1993) *Plant Physiol.* **103**, 835–843.
30. Mattoo, A. K. & Edelman, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1497–1501.
31. Klein, R. R., Mason, H. S. & Mullet, J. E. (1988) *J. Cell. Biol.* **106**, 289–301.
32. Clarke, A. K., Soitamo, A., Gustafsson, P. & Oquist, G. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 9973–9977.
33. Clarke, A. K., Hurry, V. M., Gustafsson, P. & Oquist, G. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 11985–11989.