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Rapid interchange between two distinct forms of cyanobacterial photosystem II reaction-center protein D1 in response to photoinhibition

(high light stress/protein turnover/*Synechococcus* sp. PCC 7942)

ADRIAN K. CLARKE*†, ARTO SOITAMO‡, PETTER GUSTAFSSON*, AND GUNNAR ÖQUIST*

*Department of Plant Physiology, University of Umeå, Umeå S-901 87, Sweden; and ‡Department of Biology, University of Turku, SF-20500 Turku, Finland

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ABSTRACT **We have studied photoinhibition of photosynthesis in the cyanobacterium *Synechococcus* sp. PCC 7942, which possesses two distinct forms of the photosystem II reaction-center protein D1 (D1:1 and D1:2). We report here that when cells adapted to a growth irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ are exposed to an irradiance of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the normally predominant D1 form (D1:1) is rapidly replaced with the alternative D1:2. This interchange is not only complete within the first hour of photoinhibition but is also fully reversible once cells are returned to 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. By using a mutant that synthesizes only D1:1, we show that the failure to replace D1:1 with D1:2 during photoinhibition results in severe loss of photosynthetic activity as well as a diminished capacity to recover after the stress period. We believe that this interchange between D1 forms may constitute an active component in a protection mechanism unique among photosynthetic organisms that enables cyanobacteria to effectively cope with and recover from photoinhibition.**

One of the apparent paradoxes in nature is the susceptibility of oxygenic photosynthesis to inactivation by excess light. The primary site for this high light stress, or photoinhibition, lies within the reaction center of photosystem II (PSII) which consists primarily of two structurally similar proteins, D1 and D2 (for a recent review, see ref. 1). These reaction-center proteins bind the key components that mediate primary charge separation as well as water oxidation within the thylakoid membrane. Which of these cofactors becomes irreversibly damaged during photoinhibition has yet to be resolved, although the resulting decrease in photosynthetic performance is thought to trigger the degradation of the D1 polypeptide (2, 3). D1 has an inherently fast rate of turnover which was thought to accelerate during photoinhibition (4, 5), although this response is now believed to be more variable in higher plants (6). The biosynthesis of D1 effectively copes with this unusually high rate of degradation, but under photoinhibitory conditions, the net loss of D1 from PSII supposedly exceeds the rate of D1 replacement (7). However, photoinhibition at low temperatures is at least one instance where D1 degradation does not correlate with loss in photosynthetic activity (8, 9).

The D1 protein found in plants is encoded by the *psbA* gene situated on the chloroplast genome. By contrast, all cyanobacteria studied so far possess small *psbA* multigene families that code for two distinct forms of D1 polypeptide (10–15). The genome of *Synechococcus* sp. PCC 7942 contains three *psbA* genes (*psbAI, psbAII,* and *psbAIII*) coding for two forms of D1 protein (D1:1 and D1:2) that differ in 25 of the total 360 amino acids, 12 of which occur in the first 16 amino acids (12). The *psbAI* gene codes for the D1:1 protein and is highly expressed under low and normal light conditions, normally providing >90% of the total *psbA* mRNA (12). Both *psbAII* and *psbAIII* encode the D1:2 polypeptide and are most highly expressed under high photon irradiances (12, 16).

Selective inactivation of the various *psbA* genes in *Synechococcus* sp. PCC 7942 has shown that each gene is active and capable of providing sufficient D1 protein to maintain normal photosynthesis (12). We have previously used two of these *psbA* mutants to examine the potential role of the two D1 forms in the susceptibility of *Synechococcus* sp. PCC 7942 to photoinhibition (17, 18). The mutant strain R2K1 has the *psbAI* gene inactivated by the insertion of a kanamycin-resistance cassette, whereas spectinomycin- and chloramphenicol-resistance cassettes have been used to inactivate *psbAII* and *psbAIII*, respectively, in the mutant R2S2C3 (12). We found that R2K1 (D1:2 only) was notably less susceptible to photoinhibition than R2S2C3 (D1:1 only) and that this increased resistance was due mainly to a higher rate of recovery (17, 18).

We have further investigated the susceptibility of *Synechococcus* sp. PCC 7942 to photoinhibition by examining the proportion of D1:1 and D1:2 during a moderately high light treatment, first in the wild-type strain and then in the two mutants R2K1 and R2S2C3. By use of specific antibodies that can distinguish between the two different forms of D1, we show that the normally predominant D1:1 polypeptide is rapidly replaced with D1:2 in the wild-type strain during photoinhibition. When cells are allowed to recover under the growth irradiance, the exchange between D1 forms is reversed so that D1:1 is again the major D1 species. Similar experiments with R2K1 and R2S2C3 show that this interchange between the two D1 forms is an essential component for limiting the extent of photoinhibition as well as facilitating a rapid recovery of photosynthesis in *Synechococcus* sp. PCC 7942.

MATERIALS AND METHODS

Cell Strains and Growth Conditions. *Synechococcus* sp. PCC 7942 and the two *psbA* gene-inactivated mutants R2S2C3 and R2K1 (12) were grown at 37°C under a continuous irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as detailed previously (17). Cultures were flushed with 5% CO_2 in air to avoid changes in antenna size due to low inorganic carbon content in the medium. Cells in the logarithmic phase of growth were used for all experiments.

Production of D1:1- and D1:2-Specific Polyclonal Antibodies. Antibodies specific for the two D1 forms in *Synechococcus* sp. PCC 7942 were produced by the strategy of Schaefer and Golden (19). We synthesized two oligonucleotides coding for the first 21 amino acids of the N-terminal

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Abbreviation: PSII, photosystem II.
†To whom reprint requests should be addressed.

region of both D1:1 and D1:2 and cloned them separately in frame into the *lacZ* gene from the plasmid pMC1871. These constructs were placed downstream from the *lac* promoter in the plasmid pUC8. Fusion proteins were isolated according to Schaefer and Golden (16) with the exception that cells were lysed by sonication (Branson Sonifier B15) at 4°C and Triton X-100 (0.2%) was added before ultracentrifugation. Fusion proteins were then purified on an anti-β-galactosidase affinity column (Promega). Antibodies were produced commercially (AgriSera, Vännäs, Sweden), with 50 μg of the purified fusion proteins injected into rabbits both intramuscularly and subcutaneously. Three additional injections (50–150 μg of fusion protein) were performed over a 6-week period. Serum was isolated after each injection to analyze the immune response.

Photoinhibitory Treatment and Recovery. Cells were harvested by centrifugation at 4000 × *g* for 5 min and resuspended in fresh growth medium containing 10 mM $NaHCO_3$ at a chlorophyll concentration of 5 $\mu g \cdot ml^{-1}$. All treatments were carried out in 50-ml transparent tubes in a 37°C water bath. The light source was a halogen lamp (400-W Power Star HQI-IS Osram, Berlin). The incident irradiance was measured inside the tube by using a GaAsP diode (model G1125-02, Hamamatsu) sensitive to white light only and recorded on a voltmeter (model 192, Keithley). Voltages were calibrated against a quantum sensor (Li-189, Li-Cor, Lincoln, NE) and the desired irradiance obtained with neutral density filters. Cells were bubbled with air to reduce self-shading. Before each treatment, cells were incubated for 30 min at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Photoinhibitory treatments were carried out for 2 hr at an irradiance of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The subsequent recovery period was carried out at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a further 2 hr. Controls were kept at an irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the complete 4 hr. Samples were taken at the beginning of the 4-hr control or photoinhibition/recovery periods and every 30 min thereafter.

Photosynthetic Measurements. Photosynthetic oxygen evolution was measured with a Clark-type oxygen electrode (Hansatech Instruments, Norfolk, U.K.) at the nonsaturating irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Prior to each measurement, 10 μl of 1 M $NaHCO_3$ and 10 μl of streptomycin (25 $mg \cdot ml^{-1}$) were added to each sample (2 ml) to ensure saturating CO_2 conditions and inhibit recovery dependent on *de novo* protein synthesis, respectively. Photosynthetic rates were calculated as μmol of O_2 evolved per mg of chlorophyll per hr and are presented as a percentage of the photosynthetic rate measured immediately prior to the photoinhibitory treatment.

Immunoblot Analysis. Total cellular proteins were extracted by first centrifuging samples (1 ml) at 14,000 × *g* for 5 min and then suspending the cell pellet in 120 μl of 100 mM Tris·HCl, pH 8.6/150 mM sucrose/30 mM dithiothreitol/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride/2% lithium dodecyl sulfate. Cell were disrupted by 15 sec of continuous sonication and proteins were solubilized at 75°C for 5 min. Insoluble material was removed by centrifugation at 14,000 × *g* for 5 min.

Protein samples containing equal amounts of chlorophyll (1.5 μg) were electrophoresed in lithium dodecyl sulfate/15% polyacrylamide gels (20). Proteins were transferred electrophoretically to nitrocellulose for immunoblot analysis (21). Density scanning of immunoblots was carried out with a DU-8 spectrophotometer (Beckman) by following the manufacturer's recommendations.

RESULTS

Characterization of D1:1- and D1:2-Specific Antibodies. The form specificity of the two D1 antibodies was determined with the *Synechococcus* sp. PCC 7942 wild type and the *psbA* mutant strains R2K1 and R2S2C3 (Fig. 1). Protein extracts were prepared from each strain, separated by polyacrylamide

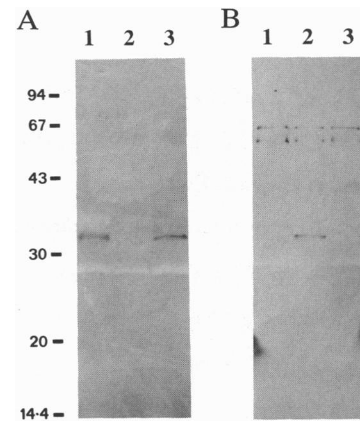

FIG. 1. Characterization of D1:1- and D1:2-specific polyclonal antibodies. The specificity of D1:1 (*A*) and D1:2 (*B*) antisera raised against the unique amino-terminal regions of each form was tested on cell extracts from the *Synechococcus* sp. PCC 7942 wild-type strain (lane 1) and the two *psbA* mutants R2K1 (lane 2) and R2S2C3 (lane 3). Each strain was grown under a continuous irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Protein extracts were electrophoresed in polyacrylamide gels containing 0.1% lithium dodecyl sulfate, with each sample loaded on the basis of equal chlorophyll (1.5 μg). After electrophoresis, proteins were transferred to nitrocellulose filters for immunoblot analysis. Filters were incubated with either primary antibody (D1:1 or D1:2) and bound antibodies were detected with the enhanced chemiluminescence system (ECL, Amersham) visualized on x-ray film.

electrophoresis, and subjected to immunoblotting using the two antibody preparations. For the mutant R2K1, which lacks D1:1 due to the inactivation of the *psbAI* gene, only the form 2-specific antibody crossreacted with the D1 protein (compare lanes 2 in Fig. 1 *A* and *B*). Conversely, only the antibodies raised against the amino terminus of D1:1 recognized the D1 protein in R2S2C3, which lacks functional *psbAII/III* genes and thus cannot produce D1:2 (compare lanes 3 in Fig. 1 *A* and *B*). The form 1-specific antibody also crossreacted with the D1 protein in the wild-type strain (Fig. 1*A*, lane 1), whereas the form 2-specific antibody produced no discernible signal (Fig. 1*B*, lane 1). It has been shown previously that when the wild-type strain of *Synechococcus* sp. PCC 7942 is grown under low irradiance, such as 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, >90% of the D1 protein is D1:1 (19). Therefore, the results in Fig. 1 clearly show that the two antibodies can be used to distinguish between D1:1 and D1:2 in *Synechococcus* sp. PCC 7942.

Interchange Between D1 Forms. The two specific antibodies were used to determine the proportion of D1:1 and D1:2 during photoinhibition and afterwards during recovery at the growth irradiance. In cells grown under an irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, D1:1 made up >95% of the total D1 protein (Fig. 2). When cells were shifted to the photoinhibitory light regime of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the previously predominant D1:1 was rapidly replaced by D1:2. This interchange between D1 forms was complete within 1 hr of the high light stress. D1:2 remained the predominant D1 form throughout the 2-hr high light treatment. Afterwards, the cells were allowed to recover for 2 hr at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During this recovery period, D1:2 was replaced with D1:1 so that D1:1 again became the predominant form. Although complete within 2 hr, this reversal in D1 forms during recovery was not as rapid as the interchange during photoinhibition.

Rate of D1:1 Degradation. The chloroplast D1 protein has an unusually high rate of turnover which supposedly accelerates with increasing irradiance (4, 5). To test whether excess light causes a similar increase in D1:1 turnover in *Synechococcus* sp. PCC 7942, we compared the rate of D1:1 degradation

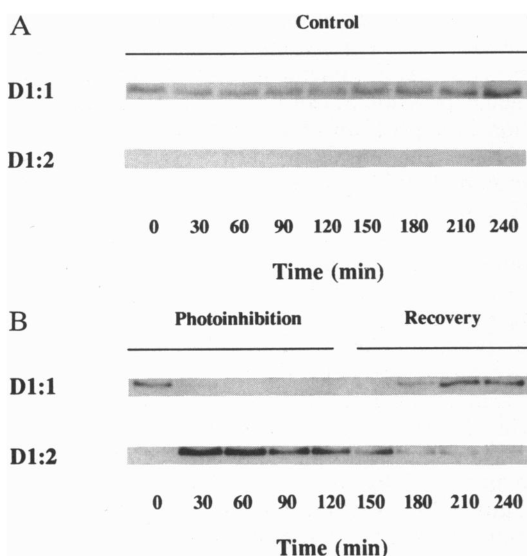

FIG. 2. Rapid interchange between D1:1 and D1:2 during photoinhibition and recovery, shown by immunodetection of D1:1 and D1:2 in cells treated under control (50 μmol·m^{-2}·s^{-1}) (*A*) or photoinhibitory/recovery (500/50 μmol·m^{-2}·s^{-1}) (*B*) light regimes. Protein extracts for D1 identification were taken every 30 min for electrophoresis in lithium dodecyl sulfate/polyacrylamide gel, with samples loaded on the basis of equal chlorophyll (1.5 μg). Detection of D1:1 and D1:2 was carried out by immunoblotting using specific antibodies as described in Fig. 1.

during photoinhibition with that at the control irradiance (Fig. 3). By adding the translation inhibitor streptomycin prior to each light treatment, the rate of D1:1 degradation was clearly shown to increase significantly at the higher irradiance. The rate of degradation at 500 μmol·m^{-2}·s^{-1} was double that at 50 μmol·m^{-2}·s^{-1} when the times taken for 50% of D1:1 to degrade were compared (Fig. 3*B*). When the residual amounts of D1:1 were compared during photoinhibition in the presence or absence of streptomycin, identical levels of D1:1 were observed, indicating that the accelerated loss of D1:1 at 500 μmol·m^{-2}·s^{-1} was not mediated by an induced protein factor. This also implied that the synthesis of D1:1 must have ceased almost immediately upon exposure to the high light stress.

Photoinhibition and Recovery in *Synechococcus* sp. PCC 7942 Mutants R2S2C3 and R2K1. In previous reports, we have used the two *psbA* inactivation mutants, R2S2C3 and R2K1, to study the physiological characteristics of cells containing PSII reaction centers comprised solely of D1:1 or D1:2, respectively (17, 18). These studies showed that R2K1 was considerably more resistant to loss of photosynthetic activity during high light stress than R2S2C3. To test whether changes in the amount of D1 could explain this difference in susceptibility to photoinhibition between the two mutants, we compared the levels of D1 in R2K1 and R2S2C3 under the same photoinhibition/recovery regime described for wild-type cells. There was a significantly greater loss of D1:1 from R2S2C3 cells than of D1:2 from R2K1 cells during photoinhibition (Fig. 4). Around 40% of D1:2 was lost from R2K1 cells after the 2-hr treatment at 500 μmol·m^{-2}·s^{-1}, after which the level of D1:2 steadily recovered to almost 100% of control values (Fig. 4*B*). By contrast, the level of D1:1 in R2S2C3 cells decreased dramatically during photoinhibition, so that no D1:1 was detected after 1 hr. D1:1 remained undetectable throughout the first hour of recovery at 50 μmol·m^{-2}·s^{-1}, and had regained only 20% of the control value after the full 2 hr (Fig. 4*B*).

Photosynthetic Measurements. When we measured the photosynthetic activity of R2K1 and R2S2C3 cells treated under identical conditions (Fig. 5), the relative extent of inactiva-

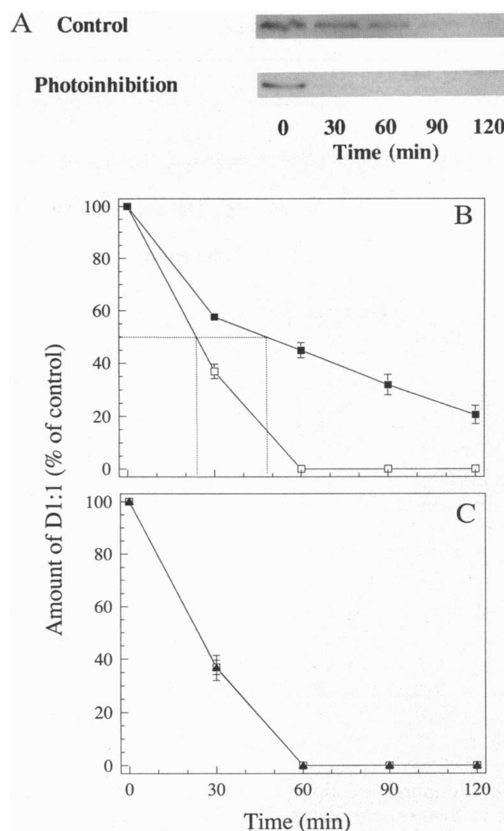

FIG. 3. Rate of D1:1 degradation during control and photoinhibitory treatments. (*A*) Immunodetection of residual D1:1 during 2 hr of treatment in the presence of streptomycin at 50 μmol·m^{-2}·s^{-1} (control) or 500 μmol·m^{-2}·s^{-1} (photoinhibition). Streptomycin (250 μg·ml^{-1}) was added 5 min before the start of each treatment. (*B*) Rate of D1:1 degradation at 50 μmol·m^{-2}·s^{-1} (■) or 500 μmol·m^{-2}·s^{-1} (□) as determined from the immunoblots shown in *A*. (*C*) Comparison between the rate of D1:1 degradation (□) and the amount of residual D1:1 (▲) during photoinhibition at 500 μmol·m^{-2}·s^{-1}. Relative amounts of D1:1 were determined by density scanning of immunoblots. Values represent means ± SD (n = 3).

tion in photosynthesis during the high light period closely matched the quantity of D1 lost for both mutants. Similarly, the degree of recovery in photosynthetic activity at 50 μmol·m^{-2}·s^{-1} corresponded to the increase in D1 for both R2K1 and R2S2C3. In comparison, the degree of inactivation of photosynthesis for the wild type was nearly identical to that for R2K1. The wild type was also capable of recovering most of its lost photosynthetic activity during the 2 hr at 50 μmol·m^{-2}·s^{-1}, although not at the same rate observed for R2K1.

DISCUSSION

In this report, we have shown that the wild-type strain of *Synechococcus* sp. PCC 7942 upon exposure to high light has the ability to rapidly and completely replace one of its two forms of PSII reaction-center protein, D1:1, with the other form, D1:2 (Fig. 2). This interchange between D1 forms during photoinhibition corresponds to the changing levels of the three *psbA* mRNA types previously observed during a similar rapid shift in irradiance. The D1:2 protein was maintained in PSII throughout the high light regime but then was rapidly exchanged for the D1:1 polypeptide once cells were allowed to recover at 50 μmol·m^{-2}·s^{-1}. In addition, the rate at which D1:1 was lost during the exchange with D1:2 during the high light treatment was identical to the degradation rate for D1:1 at 500 μmol·m^{-2}·s^{-1} (Fig. 3*C*), indicating a rapid

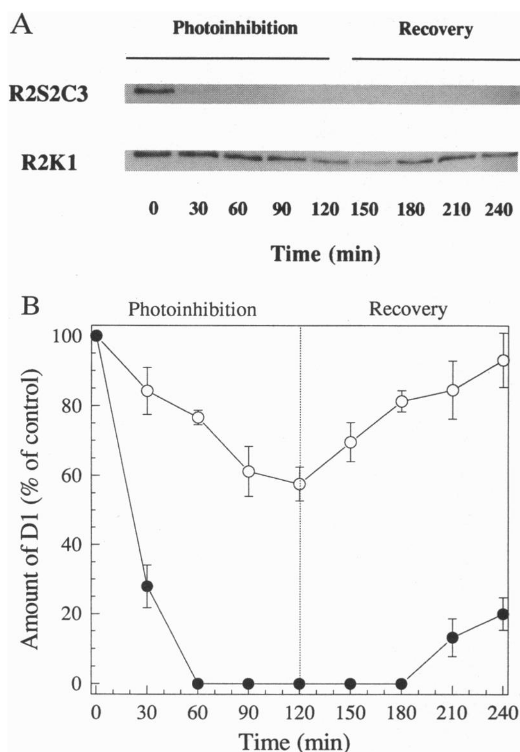

FIG. 4. Immunodetection (*A*) and quantification (*B*) of relevant D1 form in *Synechococcus* sp. PCC 7942 mutants R2S2C3 (●) and R2K1 (○) during 2 hr of photoinhibition at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ followed by 2 hr of recovery at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Experiments were carried out as described for the wild type (Fig. 2). Relative amounts of D1:1 and D1:2 were determined as detailed in Fig. 3 legend. Values represent means ± SD (n = 3).

inactivation of D1:1 synthesis. These results also show that *de novo* synthesis of a specific protease during the high light treatment is not required for the increased rate of D1:1 breakdown. This is in agreement with studies on the chloroplast D1 protein that have shown the protease involved in D1 breakdown to be an integral component of PSII (2, 22).

It is known that *Synechococcus* sp. PCC 7942 cells can regulate the level of expression for the three *psbA* genes and consequently alter the proportion between the two D1 forms when grown under varying irradiances. When grown at low irradiance, the *psbAI* mRNA makes up at least 90% of the total *psbA* message (12). For cells matured to stationary

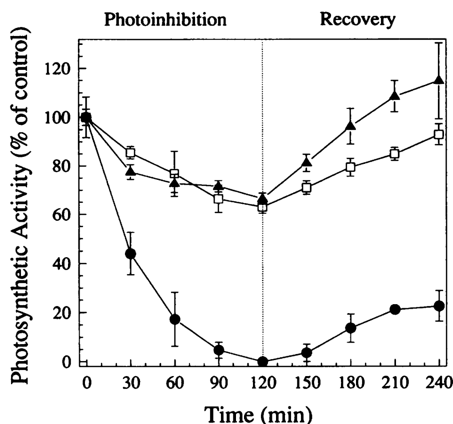

FIG. 5. Photosynthetic activity during 2 hr of photoinhibition at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, followed by 2 hr of recovery at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, in *Synechococcus* sp. PCC 7942 wild type (□) and the two mutants R2S2C3 (●) and R2K1 (▲). Values represent means ± SD (n = 3).

phase along a gradient of irradiances from 5 to 482 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the expression for a *psbAI* reporter gene decreases up to 80% concomitant with a 93% increase in the combined expression of *psbAII/III* reporter genes (16). This alteration in expression of the *psbA* genes at high light is reflected in changes in the proportion of D1 forms, with the relative amount of D1:1 in the thylakoid membranes dropping by 58% at 482 $\mu mol \cdot m^{-2} \cdot s^{-1}$ along with a 60% rise in D1:2 (19).

A more dramatic change in the expression of the three *psbA* genes occurs when *Synechococcus* sp. PCC 7942 cells are shifted from low to high irradiance. From 125 to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the levels of *psbAII/III* mRNAs increase within 15 min to around 500% of control levels. Concomitantly, transcripts from the *psbAI* gene decrease by ≈70% (23). This decrease in the *psbAI* message is partly due to a decrease in the rate of transcription of the *psbAI* gene but it is also mediated by an increased rate of *psbAI* mRNA degradation (24). The elevated rate of decay for *psbAI* transcripts was shown to be facilitated by an induced protein that may either function as a nuclease, modify the specificity of preexisting nucleases, or alter the stability of *psbA* transcripts (24). Interestingly, the *psbAIII* mRNA was also shown to decay faster at high irradiances, and the increased decay was mediated by the same putative protein factor involved in *psbAI* degradation (24).

In earlier studies characterizing the susceptibility of photosynthesis to photoinhibition in the two *psbA* mutants R2S2C3 and R2K1, we demonstrated that PSII reaction centers containing D1:2 were significantly more resistant to photoinhibition than those with only D1:1 (17, 18). We suggested that this was largely because (*i*) PSII reaction centers with D1:2 recovered more rapidly than those containing D1:1 (i.e., there was a higher capacity of the PSII repair cycle in R2K1 than in R2S2C3) and (*ii*) PSII reaction centers containing D1:2 were more intrinsically resistant to photoinhibition than those possessing D1:1. In this study, we have shown that the differential susceptibility of photosynthesis to photoinhibition in R2K1 and R2S2C3 is related primarily to their ability to sustain D1 synthesis throughout the photoinhibitory regime. This difference in susceptibility to photoinhibition between the two mutants shows clearly that the inability of R2S2C3 to induce the transcription of both *psbAII* and *psbAIII* genes results in severe loss of photosynthetic activity as well as a reduced capacity to recover once the high light stress is alleviated. Since the extent of D1:1 loss in R2S2C3 (Fig. 4*B*) matches the rate of D1:1 degradation observed in the wild type at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 3*C*), it appears that R2S2C3 also lacks the ability to sense the inactivation of *psbAII* and *psbAIII* and thus upregulate the expression of *psbAI* during photoinhibition. By contrast, inactivation of the *psbAI* gene in R2K1 results in the induction of *psbAII* and *psbAIII* expression, so that R2K1 grows only 10% slower than the wild type at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (18). The regulatory mechanism that enables this enhanced level of *psbAII* and *psbAIII* expression at low irradiance is unknown.

When we compared the loss in photosynthetic activity in the wild type with that of the two mutants, the degree of susceptibility to photoinhibition in the wild type could be seen to correspond to that of R2K1. This similarity reflected the rapid interchange of D1 forms observed in the wild-type cells and that D1:2 became the sole form of D1 within 1 hr of high light treatment. During the recovery period, however, the wild type recovered photosynthetic activity at a slower rate than R2K1. We believe that this was due to the replacement of D1:2 by D1:1 that occurred in the wild type and that its rate of recovery was limited by this second interchange between D1 forms at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

The underlying photochemical processes within PSII that are damaged during photoinhibition are yet to be resolved. It is thought that absorption of excess excitation energy leads to the formation of highly oxidizing radicals and toxic oxygen species within the reaction center (22). The increased rate of D1 breakdown, therefore, is believed to be a consequence of this increased rate of radical formation (2, 3). Photoinhibition results primarily when the rate of D1 resynthesis fails to cope with the increasing rate of damaged D1 formed within PSII (1). We propose that the rapid replacement of D1:1 by D1:2 in *Synechococcus* sp. PCC 7942 is a unique protective mechanism for limiting the severity of photoinhibition as well as facilitating a rapid return to normal photosynthetic activity. One part of this mechanism is the raised level of expression for the *psbAII/III* genes, leading to an enhanced synthesis of D1 in the form of D1:2. The additional D1 protein may be required in part to compensate for the increased rate of D1 degradation during photoinhibition. Interestingly, *Synechococcus* sp. PCC 7942 also possesses two *psbD* genes, which code for identical copies of the other PSII reaction-center polypeptide, D2 (25). One of these genes, *psbDI*, is expressed constitutively, whereas *psbDII* is induced mainly at high irradiances (26). It is possible that the additional synthesis of D2 also compensates for an increased rate of D2 degradation. Thus, by supplementing the synthesis of both D1 and D2 during photoinhibition, the severity of damage to PSII may be reduced and consequently enable a more rapid recovery.

The fact that small *psbA* multigene families that code for two different forms of D1 have been found in all genera of cyanobacteria examined (10–13, 15) suggests that such a protective mechanism may be a common feature for cyanobacteria. Such a mechanism, however, is unique among photosynthetic organisms, since plants possess only a single form of D1 protein, and thus presumably additional strategies are necessary for limiting the extent of photoinhibition. In higher plants, the intrinsic susceptibility of PSII appears to be very constant among species when comparisons are made under similar excitation pressures as set by the redox state of the primary, stable electron acceptor Q_A (27). Resistance to photoinhibition in these organisms is related to control of energy absorption (28), energy dissipation (29), and energy distribution (30). Similar mechanisms also appear to operate in *Synechococcus* sp. PCC 7942 (31), although *Synechococcus* sp. PCC 7942 also has the ability to modulate resistance to photoinhibition at the reaction center by interchange of D1:1 with D1:2.

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1. Barber, J. & Andersson, B. (1992) *Trends Biochem. Sci.* **17,** 61–66.
2. Shipton, C. A. & Barber, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6691–6695.
3. Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.-M. & Andersson, B. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1408–1412.
4. Mattoo, A. K., Pick, U., Hoffman-Falk, H. & Edelman, M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1572–1576.
5. Kyle, D. J., Ohad, I. & Arntzen, C. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4070–4074.
6. Sundby, C., McCaffery, S., Chow, W. S. & Anderson, J. M. (1993) in *Current Research in Photosynthesis*, ed. Murata, N. (Kluwer, Dordrecht, The Netherlands), Vol. 4, pp. 443–446.
7. Kyle, D. J. (1987) in *Photoinhibition*, eds. Kyle, D. J., Osmond, C. B. & Arntzen, C. J. (Elsevier, Amsterdam), pp. 197–226.
8. Chow, W. S., Osmond, C. B. & Huang, L. K. (1989) *Photosynth. Res.* **21,** 17–26.
9. Öttander, C., Hundal, T., Andersson, B., Huner, N. P. A. & Öquist, G. (1993) *Photosynth. Res.* **35,** 191–200.
10. Curtis, S. E. & Haselkorn, R. (1984) *Plant Mol. Biol.* **3,** 249–258.
11. Mulligan, B., Schultes, N., Chen, L. & Bogorad, L. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2693–2697.
12. Golden, S. S., Brusslan, J. & Haselhorn, R. (1986) *EMBO J.* **5,** 2789–2798.
13. Jansson, C., Debus, R. J., Osiewacz, H. D., Gurevitz, M. & McIntosh, L. (1987) *Plant Physiol.* **85,** 1021–1025.
14. Gingrich, J. C., Buzby, J. S., Stirewalt, V. L. & Bryant, D. A. (1988) *Photosynth. Res.* **16,** 83–99.
15. Bouyoub, A., Vernotte, C. & Astier, C. (1993) *Plant Mol. Biol.* **21,** 249–258.
16. Schaefer, M. R. & Golden, S. S. (1989) *J. Bacteriol.* **171,** 3973–3981.
17. Krupa, Z., Öquist, G. & Gustafsson, P. (1990) *Plant Physiol.* **93,** 1–6.
18. Krupa, Z., Öquist, G. & Gustafsson, P. (1991) *Physiol. Plant.* **82,** 1–8.
19. Schaefer, M. R. & Golden, S. S. (1989) *J. Biol. Chem.* **264,** 7412–7417.
20. Clarke, A. K. & Critchley, C. (1990) *Plant Physiol.* **94,** 567–576.
21. Clarke, A. K. & Critchley, C. (1992) *Plant Physiol.* **100,** 2081–2089.
22. Tefler, A., De Las Rivas, J. & Barber, J. (1991) *Biochim. Biophys. Acta* **1060,** 106–114.
23. Bustos, S. A., Schaefer, M. R. & Golden, S. S. (1990) *J. Bacteriol.* **172,** 1998–2004.
24. Kulkarni, R. D., Schaefer, M. R. & Golden, S. S. (1992) *J. Bacteriol.* **174,** 3775–3781.
25. Golden, S. S., Cho, D.-S. C. & Nalty, M. S. (1989) *J. Bacteriol.* **171,** 4707–4713.
26. Bustos, S. A. & Golden, S. S. (1992) *Mol. Gen. Genet.* **232,** 221–230.
27. Öquist, G., Chow, W. S. & Anderson, J. M. (1992) *Planta* **186,** 450–460.
28. Anderson, J. M. & Osmond, C. B. (1987) in *Photoinhibition*, eds. Kyle, D. J., Osmond, C. B. & Arntzen, C. J. (Elsevier, Amsterdam), pp. 1–38.
29. Krause, G. H. & Weis, E. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42,** 313–349.
30. Horton, P. (1989) in *Photosynthesis*, ed. Briggs, W. R. (Liss, New York), pp. 393–406.
31. Samuelsson, G., Lönneborg, A., Gustafsson, P. & Öquist, G. (1987) *Plant Physiol.* **83,** 438–441.