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Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage–repair cycle of Photosystem II in *Synechocystis* sp. PCC 6803

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Abstract

The Photosystem II complex (PSII) is susceptible to inactivation by strong light, and the inactivation caused by strong light is referred to as photoinactivation or photoinhibition. In photosynthetic organisms, photoinactivated PSII is rapidly repaired and the extent of photoinactivation reflects the balance between the light-induced damage (photodamage) to PSII and the repair of PSII. In this study, we examined these two processes separately and quantitatively under stress conditions in the cyanobacterium *Synechocystis* sp. PCC 6803. The rate of photodamage was proportional to light intensity over a range of light intensities from 0 to 2000 μE m^{-2} s^{-1}, and this relationship was not affected by environmental factors, such as salt stress, oxidative stress due to H_2O_2, and low temperature. The rate of repair also depended on light intensity. It was high under weak light and reached a maximum of 0.1 min^{-1} at 300 μE m^{-2} s^{-1}. By contrast to the rate of photodamage, the rate of repair was significantly reduced by the above–mentioned environmental factors. Pulse-labeling experiments with radiolabeled methionine revealed that these environmental factors inhibited the synthesis de novo of proteins. Such proteins included the D1 protein which plays an important role in the photodamage–repair cycle. These observations suggest that the repair of PSII under environmental stress might be the critical step that determines the outcome of the photodamage–repair cycle.

Keywords: D1 protein; Photodamage and repair; Environmental stress; Photosystem II; *Synechocystis* PCC6803

1. Introduction

Light is essential for photosynthesis in plants but it can also be toxic to the photosynthetic machinery ([1] for reviews, see Refs. [2,3]). Exposure to strong visible light results in inactivation of the Photosystem II complex (hereafter, PSII) and, in particular, damage to the D1 protein in the photochemical reaction center of PSII [4–7]. This phenomenon is referred to as "photoinhibition" or "photoinactivation" and has been studied extensively in vitro, for example, in isolated thylakoid membranes [3,7].

In intact photosynthetic organisms, photoinactivation is a more complex phenomenon than it is in vitro. The extent of photoinactivation of PSII is a result of the balance between the light-induced damage (photodamage) to PSII and the repair of photodamaged PSII [3]. Photodamaged PSII is repaired by replacement of damaged D1 protein by newly synthesized D1 [3–6,8]. The damaged D1 protein is cleaved at a site in the stromal D–E loop by DegP2, a specific endoprotease [9], and is degraded by a metalloprotease, designated FtsH [10], for removal from PSII. At this point, it seems likely that PSII might be disassembled to some extent and then D1-depleted PSII is repaired by the introduction of newly synthesized D1 protein [3–6,8,11]. After PSII has been reassembled, with the incorporation of the newly synthesized D1 protein, the complex is once again capable of functioning in the photosynthetic transport of electrons.

The molecular mechanisms for photodamage and repair of PSII in cyanobacteria are similar to those in plants

Abbreviations: BQ, 1,4-benzoquinone; Chl, chlorophyll; PSII, Photosystem II

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[12–14]. Cyanobacteria are particularly useful for studies of photodamage and repair because the techniques of molecular genetics can easily be applied to these microorganisms and, in particular, to autotransformable strains, such as *Synechocystis* sp. PCC 6803 (hereafter, *Synechocystis*), *Synechococcus* sp. PCC 7942 and *Synechococcus* sp. PCC 7002 [15]. Using these microorganisms, it is possible to characterize individual steps in the photodamage–repair cycle in greater detail than is possible at present in plants. For example, Silva et al. [16] separately mutated four *ftsH* genes for the FtsH protease and characterized the role of this protease in the photodamage–repair cycle. Moreover, there are three genes for DegP, the other protease that is important for the degradation of D1, in the *Synechocystis* genome (see Cyanobase database; Ref. 17), and a genetic approach to the characterization of this enzyme has been initiated [18]. The role of the turnover of D1 and D2 in the photodamage–repair cycle has been investigated in *Synechocystis* and mutant cells have been analyzed that contain various types and various levels of *psbA* transcripts [19].

The efficiency of photosynthesis is affected by many types of environmental stress, such as a high concentration of salt, abnormal levels of reactive oxygen species, and unusually high or low temperature [2,20–23]. These types of stress appear to inactivate the photosynthetic machinery itself and the machinery that regulates photosynthesis [2,3]. In natural habitats, photosynthetic organisms are exposed to combinations of various stresses, and it is possible that the photosynthetic machinery might be inactivated to a considerable degree by exposure to combinations of two or more types of stress. Even if each individual stress is insufficient to cause damage, a combination might cause serious damage as a result of the synergistic effects of the various stresses [24,25].

Visible light might play two roles, inducing damage to PSII when it is strong and inducing repair of the photodamaged PSII when it is weak [3,26]. The repair process is the most important mechanism by which plant cells maintain functional photosynthetic machinery during and after exposure to light stress. However, there has been no systematic and quantitative analysis of such photodamage and repair when some additional environmental stress is combined with light stress. Published results related to light-induced photodamage to PSII in the presence of other types of stress have been inconsistent: for example, several research groups have suggested that oxidative stress accelerates photodamage because it inactivates the D1 protein directly [27–30], whereas other groups have reported that oxidative stress appears to enhance photoinactivation because it inhibits the repair of PSII [24]. These discrepancies in discussions of the mechanisms responsible for the synergistic effects of environmental stress might originate from the absence of systematic and quantitative analysis of the effects of environmental stress on the photodamage and repair cycle.

Cyanobacteria are very useful model organisms for analyses of the synergistic effects of various kinds of stress on the repair of PSII since it is possible to control the intensities of two or more kinds of stress much more precisely in suspension cultures of cyanobacteria than in plant leaves. For example, the use of *Synechocystis* cells has allowed us to modulate various kinds of stress simultaneously to investigate the mechanisms of their effects on the photodamage–repair cycle. Thus, we have demonstrated in previous studies that repair, but not photodamage, is affected by oxidative stress [24], salt stress [25], and cold stress [31,32].

In the present study, we examined the effects of various types of environmental stress, such as a high concentration of salt, a high level of reactive oxygen species and low temperature, on photodamage to PSII and the repair of photodamaged PSII by studying each process separately and quantitatively in *Synechocystis*. We found that the rate of photodamage was unaffected by the environmental factors, other than light, that we examined. We found, in addition, that the rate of repair also depended on light intensity but reached a maximum under relatively weak light, such as light at 300 μE m^{-2} s^{-1}, while, by contrast to photodamage, repair was severely inhibited by each type of environmental stress examined. Labeling of proteins in vivo demonstrated that these environmental factors inhibited the synthesis of the D1 protein de novo.

2. Materials and methods

2.1. Cyanobacterial strain and culture conditions

Synechocystis sp. PCC 6803 was kindly donated by Dr. J.G.K. Williams of DuPont de Nemours and Co. (Wilmington, DE). Cells were grown photoautotrophically in glass tubes (2.5 cm i.d. × 20 cm; 120 ml) at 34 °C under constant illumination from incandescent lamps at 70 μE m^{-2} s^{-1} (in which E indicates an einstein, namely, 1 mol of photons) in BG-11 medium [33] supplemented with 20 mM HEPES-NaOH (pH 7.5). This medium contained 20 mM NaCl. Cultures were aerated with sterile air that contained 1% (v/v) CO_2 [34].

2.2. Conditions for photodamage and repair

Cells from 3-day-old cultures, at a chlorophyll (Chl) concentration of 3 μg ml^{-1}, were incubated in strong light to induce photoinactivation or in weak light to induce repair in growth chambers at 34 °C under the same conditions as described above. Small aliquots of cultures were withdrawn at designated times for measurements of PSII activity. Strong light for photoinactivation was provided by two incandescent lamps (300 W; Toshiba, Tokyo, Japan), and it was attenuated to 250, 500, 750, 1000, 1500 and 2000 μE m^{-2} s^{-1} by passage through appropriate

neutral-density filters (PC-S380; 3 × 200 × 520 mm; Hoya Glass, Tokyo, Japan). The intensity of light was measured at the surface of the glass tubes that contained suspensions of cells. The repair of PSII was allowed to proceed under weak light at intensities from 50 to 1000 μE m^{-2} s^{-1}, which was provided by the same lamps as mentioned above in combination with neutral-density filters, after the activity of PSII had been reduced to 10% of the original level by strong light. In some experiments, the synthesis of proteins de novo was inhibited by lincomycin (Sigma Chemical Co., St. Louis, MO), which was added to the culture medium at 250 μg ml^{-1} 10 min before the start of incubation.

2.3. Measurement of photosynthetic activity

The activity of PSII in intact cells was measured by monitoring oxygen-evolving activity in the presence of 1.0 mM 1,4-benzoquinone (BQ), as an artificial acceptor of electrons, with a Clark-type oxygen electrode (Hansatech Instruments, King′s Lynn, UK). The sample, in a 3-ml cuvette, was illuminated by light from an incandescent lamp that had been passed through a red optical filter (R-60; Toshiba) and an infrared-absorbing filter (HA-50; Hoya Glass). The intensity of light at the surface of the cuvette was 2000 μE m^{-2} s^{-1}. Concentrations of Chl were determined as described by Arnon et al. [35].

2.4. Labeling of proteins in vivo

Synechocystis cells at a concentration corresponding to 3.00 ± 0.05 μg Chl ml^{-1} were incubated for 110 min, in the absence of lincomycin, in growth chambers at 34 °C, in light at 2000 μE m^{-2} s^{-1}, to induce 90% inactivation of PSII. Then the medium was supplemented with 10 nM [^{35}S]methionine (>1000 Ci $mmol^{-1}$; Amersham Pharmacia Biotech, Buckinghamshire, UK), as described previously [24,25], and cells were incubated for designated periods of time in light at 300 μE m^{-2} s^{-1} under control and stress conditions, as indicated. The labeling was terminated by the addition of non-radioactive methionine to a final concentration of 1.0 mM and immediate cooling of samples on ice. Cells were collected by centrifugation at 5000 × *g* for 6 min at 4 °C, and thylakoid membranes were isolated from these cells as described previously [36]. Thylakoid membranes were solubilized by incubation for 5 min at 65 °C in 60 mM Tris–HCl (pH 6.8) that contained 2% (w/v) sodium dodecyl sulfate, 5% (v/v) 2-mercaptoethanol and 10% (v/v) glycerol, and then proteins were separated by polyacrylamide gel electrophoresis [12.5% (w/v) polyacrylamide] in the presence of 0.08% (w/v) sodium dodecyl sulfate and 6 M urea, as described previously [37,38]. Solubilized thylakoid membranes corresponding to 0.8 μg of Chl *a* were loaded in each 3-mm-wide lane. Labeled proteins on the gel were visualized by exposure of the dried and fixed gel to X-ray film. Radioactivity of radiolabeled D1 was quantitated with a digital camera system (LAS-1000; Fuji Photo Film Co., Tokyo, Japan).

3. Results

3.1. Photoinactivation of PSII and the effects of lincomycin and light intensity

Fig. 1A shows the effects of light intensity on the photoinactivation of PSII, as monitored in terms of the photosynthetic evolution of oxygen with BQ as the artificial acceptor of electrons. No inactivation of PSII was apparent when *Synechocystis* cells were exposed to light at 500 μE m^{-2} s^{-1} for 90 min. This observation suggests that repair occurred more rapidly than photodamage at this light

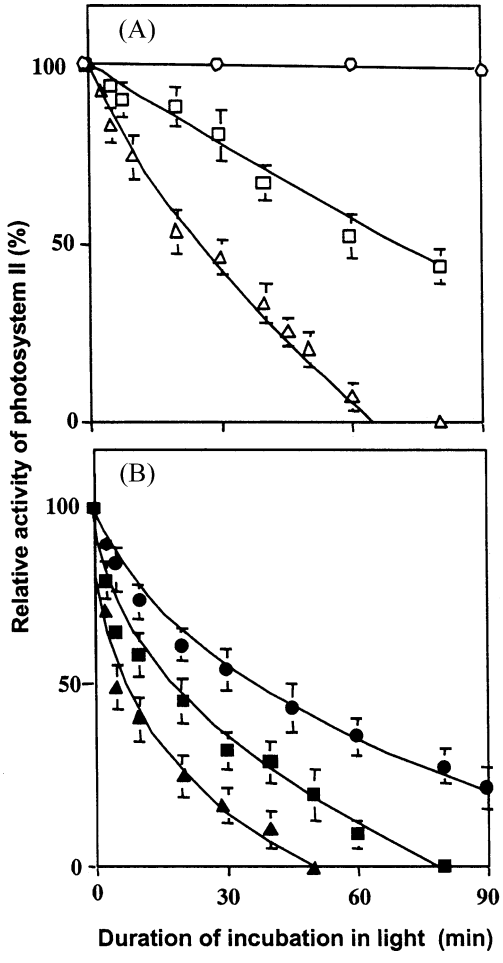

Fig. 1. Effects of light intensity and lincomycin on the photoinactivation of PSII in *Synechocystis*. (A) No addition (open symbols); (B) in the presence of 250 μg ml^{-1} lincomycin (closed symbols). ○ and ●, 500 μE m^{-2} s^{-1}; □ and ■, 1000 μE m^{-2} s^{-1}; △ and ▲, 2000 μE m^{-2} s^{-1}. At designated times, aliquots of each suspension of cells were withdrawn and PSII activity was measured, in terms of the photosynthetic evolution of oxygen, in the presence of 1.0 mM BQ as the electron acceptor. The activity that corresponded to 100% was 512 ± 48 μmol O_2 mg^{-1} Chl h^{-1}. Each point and bar represent the average ± S.E. of results from four independent experiments.

intensity. However, when the duration of incubation at 500 μE m^{-2} s^{-1} was extended beyond 90 min, the activity of PSII declined gradually and reached a plateau value of 90% of the original. At 1000 μE m^{-2} s^{-1} inactivation was observed from the start of the incubation and, after incubation for 60 min, the PSII activity had fallen to about 50% of the original value. When the duration of incubation was extended to 180 min, the PSII activity fell to 30% of the original value and remained at this level for up to 240 min. This observation suggests that, at this light intensity, photodamage and repair almost balanced each other out. At 2000 μE m^{-2} s^{-1}, the inactivation of PSII occurred much more rapidly and, after incubation of cells for 60 min, PSII activity had almost disappeared, suggesting that photodamage had occurred more rapidly than repair.

To examine photodamage independently of repair, we examined the photoinactivation of PSII in the presence of 250 μg ml^{-1} lincomycin, which inhibits protein synthesis and, thus, the repair of PSII [39]. The results in Fig. 1B show that lincomycin significantly accelerated the photoinactivation of PSII. In particular, at 500 μE m^{-2} s^{-1}, PSII activity disappeared after 180 min, in marked contrast to the results obtained in the absence of lincomycin. At 1000 μE m^{-2} s^{-1}, PSII activity had disappeared after incubation for 80 min. At 2000 μE m^{-2} s^{-1}, PSII activity disappeared after incubation for only 50 min, implying that inactivation of PSII occurred much more rapidly in the presence of lincomycin than in its absence.

To investigate the relationship between light intensity and the extent of photodamage, we determined the initial rate of photoinactivation of PSII under light at various intensities. Fig. 2 shows that, when examined in terms of the initial rate, inactivation in the absence of lincomycin did not become evident until the intensity of light was raised to 1000 μE m^{-2} s^{-1}. In the presence of lincomycin, there was no such lag phase, and the initial rate of photoinactivation (or photodamage) was directly proportional to light intensity at all intensities examined. Similar results have been observed in leaves of higher plants that were treated with lincomycin [39,40].

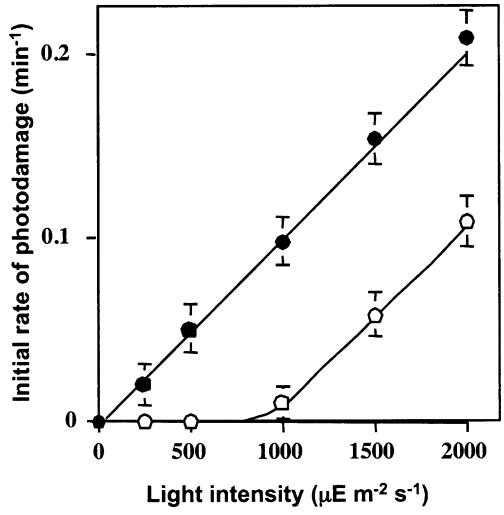

Fig. 2. Effects of light intensity on the initial rate of photoinactivation of PSII. Cells were exposed to light at various intensities at 34°C in the presence and absence of 250 μg ml^{-1} lincomycin and the initial rates of photoinactivation were determined from the time courses of photoinactivation, as described in Materials and methods. ○, No addition; ●, in the presence of 250 μg ml^{-1} lincomycin. Each point and bar represent the average ± S.E. of results from six independent experiments.

Table 1
Effects of NaCl, H_2O_2 and temperature on the initial rate of photodamage to PSII in the presence of lincomycin

Conditions	Light intensity (μE m^{-1} s^{-1})	
	1000 $(min)^{-1}$	2000 $(min)^{-1}$
Control	0.090 ± 0.007	0.194 ± 0.009
NaCl (M)		
0.5	0.102 ± 0.007	0.193 ± 0.010
1.0	0.097 ± 0.007	0.208 ± 0.006
H_2O_2 (mM)		
0.5	0.107 ± 0.008	0.212 ± 0.008
1.0	0.091 ± 0.008	0.190 ± 0.009
Temperature (°C)		
34	0.100 ± 0.009	0.200 ± 0.008
25	0.101 ± 0.005	0.197 ± 0.008
18	0.092 ± 0.008	0.202 ± 0.007
10	0.099 ± 0.006	0.201 ± 0.008

Synechocystis cells that had been grown in light at 70 μE m^{-2} s^{-1} were exposed to light at 1000 or 2000 μE m^{-2} s^{-1} in the presence of 250 μg ml^{-1} lincomycin under various conditions, as indicated, and the initial rate of photodamage to PSII was determined from the time course of each individual experiment. Results are means ± S.E. of results from five assays in each case.

3.2. The rate of photodamage to PSII is unaffected by environmental stress

Various types of environmental stress, such as salt stress, oxidative stress and low-temperature stress, accelerate the photoinactivation of PSII [2,27–30,41,42]. We investigated whether these three types of stress might accelerate photoinactivation in the presence of lincomycin, which, by inhibiting repair, allows us to monitor photodamage exclusively. Table 1 shows that the initial rate of photoinactivation was unaffected by NaCl at various concentrations. Oxidative stress due to the addition of exogenous H_2O_2 also had no effect on the rate of photodamage. As shown also in Table 1, the rate of photodamage was unaffected by low temperature under these conditions.

3.3. Effects of light intensity on the repair of PSII

As shown in Fig. 3, the PSII activity of *Synechocystis* cells that had been exposed to strong light at 2000 μE m^{-2} s^{-1} for 110 min, to reduce the activity of PSII to 90% of the original level, was restored during exposure to weak light at various intensities. At 50 μE m^{-2} s^{-1}, the rate of recovery was low but, after incubation for 90 min, PSII activity had returned to the original level. At 300 μE m^{-2} s^{-1}, the rate

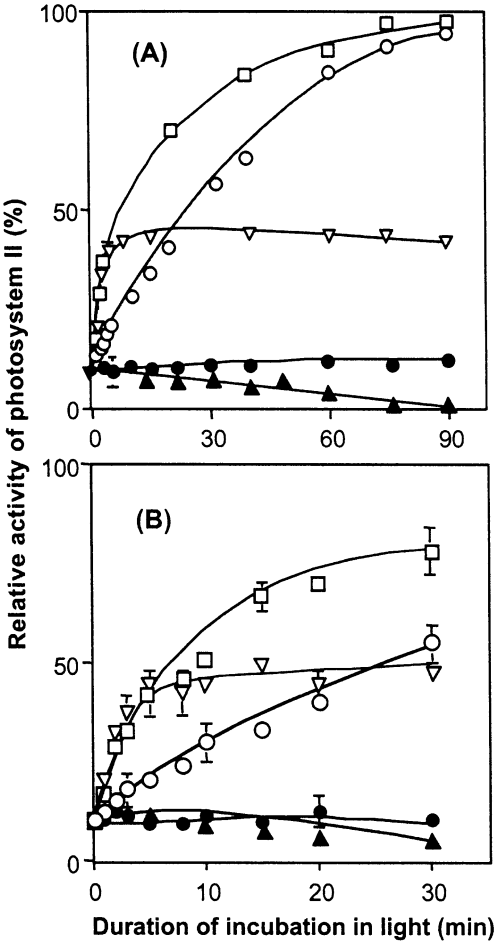

Fig. 3. Effects of light intensity on the recovery of PSII activity from photoinactivation in *Synechocystis*. Cells were incubated for 110 min, in the absence of lincomycin, in light at 2000 μE m^{-2} s^{-1} to induce 90% inactivation of PSII. Then cells were incubated in light at various intensities to allow recovery of PSII activity. Panels (A) and (B) show the results of the same experiment with different time scales. Light intensity (in μE m^{-2} s^{-1}): ○, 70; □, 300; and ▽, 1000. ●, Darkness; ▲, in the presence of 250 μg ml^{-1} lincomycin in light at 300 μE m^{-2} s^{-1}. Each point and bar represent the average ± S.E. of results from five independent experiments.

of recovery was high and the activity had returned to the original level after incubation for 90 min. With further increases in light intensity to 600 or 1000 μE m^{-2} s^{-1}, there was no further increase in the rate of recovery and, during incubation for 20 min, PSII activity reached a plateau value that was much lower than the original value. It is likely that the rate of photodamage and the rate of repair balanced each other out under light at these intensities.

Fig. 4 shows the effects of light intensity on the initial rate of recovery of PSII activity during the repair of PSII. The initial rate of recovery reached a "saturation-type" curve when plotted against light intensity, with maximum rate of 0.1 min^{-1}, which corresponded to 10% recovery of PSII activity in 1 min, at approximately 300 μE m^{-2} s^{-1}. The initial rate was about half of the maximum rate at approximately 70 μE m^{-2} s^{-1}. These results suggest that there might be a reaction that limits the rate of the total repair process and that the maximum turnover rate of this reaction was 0.1 min^{-1} under our experimental conditions. The initial rates of photodamage and repair were the same at a light intensity of approximately 900 μE m^{-2} s^{-1} (Fig. 4). In a separate experiment, we found that when cells were grown at 150 μE m^{-2} s^{-1}, namely, at a little more than twice the intensity of light that corresponds to our standard conditions, the maximum rate of repair also doubled, reaching 0.2 min^{-1} at approximately 500 μE m^{-2} s^{-1} (data not shown).

3.4. Inhibition of the repair of photodamaged PSII by environmental stress

Next, we examined the inhibition of repair by NaCl by monitoring the recovery of PSII activity after photodamage (Fig. 5A). Incubation of cells in light at 2000 μE m^{-2} s^{-1} for 110 min decreased the activity of PSII to approximately 10% of the original value. Then, to monitor the recovery of photodamaged PSII, we incubated the cells in light at different intensities in the presence of NaCl at various concentrations. As shown in Fig. 5A, the initial rate of recovery was depressed by 50% by 0.5 M NaCl and recovery reached a maximum at 200 μE m^{-2} s^{-1}. Recovery was completely inhibited by NaCl at 1.0 M. Fig. 5A also shows that the light intensity at which photodamage and repair were in equilibrium shifted from approximately 900 to 500 μE m^{-2} s^{-1} in the presence of 0.5 M NaCl.

We also studied the effects of H_2O_2 on the repair of photodamaged PSII. As shown in Fig. 5B, exogenous H_2O_2 at 0.5 mM decreased the ability of cells to repair photo-

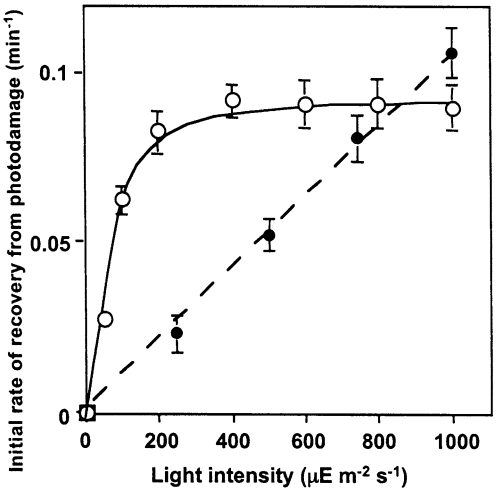

Fig. 4. Effects of light intensity on the initial rate of recovery of PSII activity from photoinactivation in *Synechocystis*. Cells were incubated, in the absence of lincomycin, in light at 2000 μE m^{-2} s^{-1} to induce 90% inactivation of PSII. Then cells were incubated in light at various intensities. The initial rates of recovery were calculated from the time courses of recovery experiments. The dashed line indicates the initial rate of photodamage to PSII in the presence of lincomycin (see Fig. 2). Each point and bar represent the average ± S.E. of results from six independent experiments.

damaged PSII and the initial rate of recovery was depressed to approximately 30% of the maximum initial rate. The intensity of light at which photodamage and repair were in complete equilibrium shifted from 900 to 300 μE m^{-2} s^{-1}. Furthermore, the initial rate of recovery fell to zero in the presence of 1.0 mM H_2O_2.

Low temperature was also effective in inhibiting the repair of photodamaged PSII. Fig. 5C shows the dependence on light intensity of the initial rate of repair at various temperatures. When the temperature was decreased from 34 to either 25 or 18 °C, the initial rate of recovery was reduced to approximately 50% and 20% of the original rate, respectively. Moreover, repair was almost completely inhibited at 10 °C. Fig. 5C also shows that the intensity of light at which photodamage and repair of PSII were in equilibrium shifted from 900 μE m^{-2} s^{-1} at 34 °C to 500 μE m^{-2} s^{-1} and 200 μE m^{-2} s^{-1} at 25 °C and 18 °C, respectively.

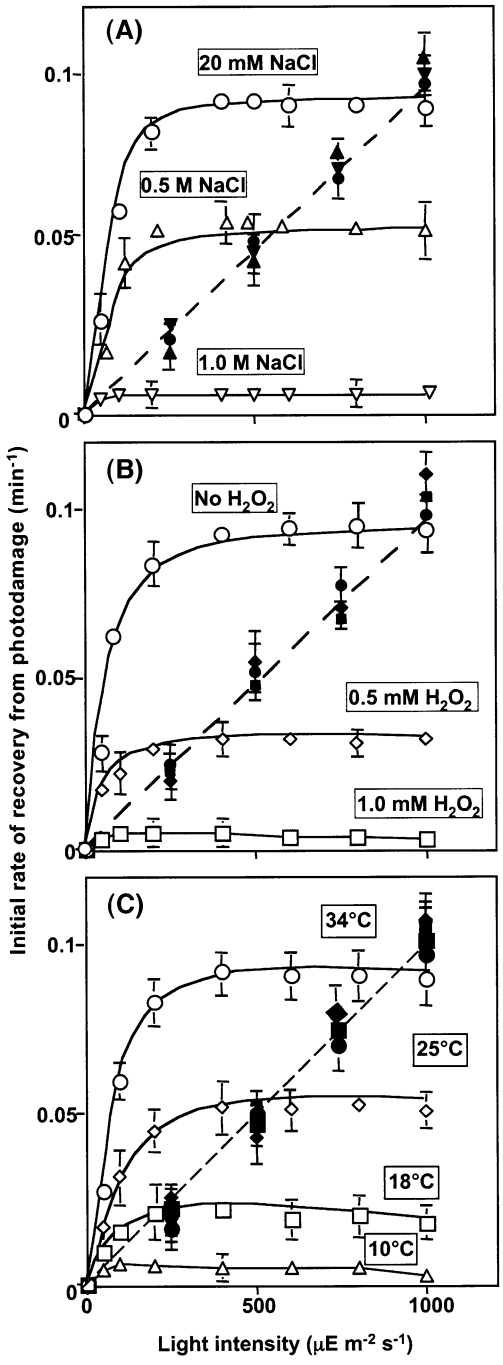

Fig. 5. Effects of light intensity, NaCl (A), H_2O_2 (B) and temperature (C) on the initial rate of recovery of PSII activity from photoinactivation in *Synechocystis*. Cells were incubated, in the absence of lincomycin, in light at 2000 μE m^{-2} s^{-1} to induce 90% inactivation of PSII. Then cells were incubated in light at various intensities in the presence and absence of NaCl, H_2O_2 and at various temperatures. Initial rates of recovery were calculated from the time courses of recovery experiments. (A) ○, 20 mM NaCl; △ and ▽, in the presence of 0.5 M and 1.0 M NaCl, respectively. The dashed line indicates the initial rate of photodamage to PSII in the presence of lincomycin plus 0.5 M NaCl (▲), plus 1.0 M NaCl (▼) and without NaCl (●) (see Fig. 2). Each point and bar represent the average ± S.E. of results from four independent experiments. (B) ○, No addition; ◇ and □, in the presence of 0.5 mM and 1.0 mM H_2O_2, respectively. The dashed line indicates the initial rate of photodamage to PSII in the presence of lincomycin plus 0.5 mM H_2O_2 (◆), plus 1.0 mM H_2O_2 (■) and without H_2O_2 (●) (see Fig. 2). Each point and bar represent the average ± S.E. of results from five independent experiments. (C) The initial rates of recovery at 34 °C (○), 25 °C (◇), 18 °C (□) and 10 °C (△). The dashed line indicates the initial rate of photodamage to PSII in the presence of lincomycin at 34 °C (●), 25 °C (◆), 18 °C (■) and 10 °C (▲). Each point and bar represent the average ± S.E. of results from five independent experiments.

3.5. Inhibition of protein synthesis by environmental stress

We studied the effects of NaCl on protein synthesis de novo by monitoring the incorporation of [^{35}S]Met into the proteins of thylakoid membranes under recovery conditions. As shown in Fig. 6A, NaCl at 0.5 M significantly suppressed the synthesis of many proteins and, as indicated in Fig. 6B, the rate of synthesis of D1 was reduced to about 50% of that in the control. Furthermore, NaCl at 1.0 M completely inhibited the synthesis of all the thylakoid proteins. Fig. 6A demonstrates, in addition, that not only the synthesis of the D1 protein but also the synthesis of many other proteins was completely inhibited by the presence of 1.0 M NaCl.

We also examined the action of H_2O_2 on the synthesis of proteins de novo. Fig. 7 shows details of the incorporation of [^{35}S]Met into proteins and, in particular, into the D1 protein. The synthesis of D1 was markedly suppressed in the presence of 0.5 mM H_2O_2. Furthermore, not only the synthesis of the D1 protein but also the synthesis of other proteins in the thylakoid membranes was inhibited by the presence of 0.5 mM H_2O_2. The synthesis of all proteins in the thylakoid membranes was totally blocked in the presence of 1.0 mM H_2O_2.

To examine the effects of low temperature on the synthesis of proteins during the repair of PSII, we incubated photoinhibited cells, which had been incubated at 34 °C in light at 2000 μE m^{-2} s^{-1}, with [^{35}S]Met in light at 300 μE m^{-2} s^{-1} at various temperatures. As

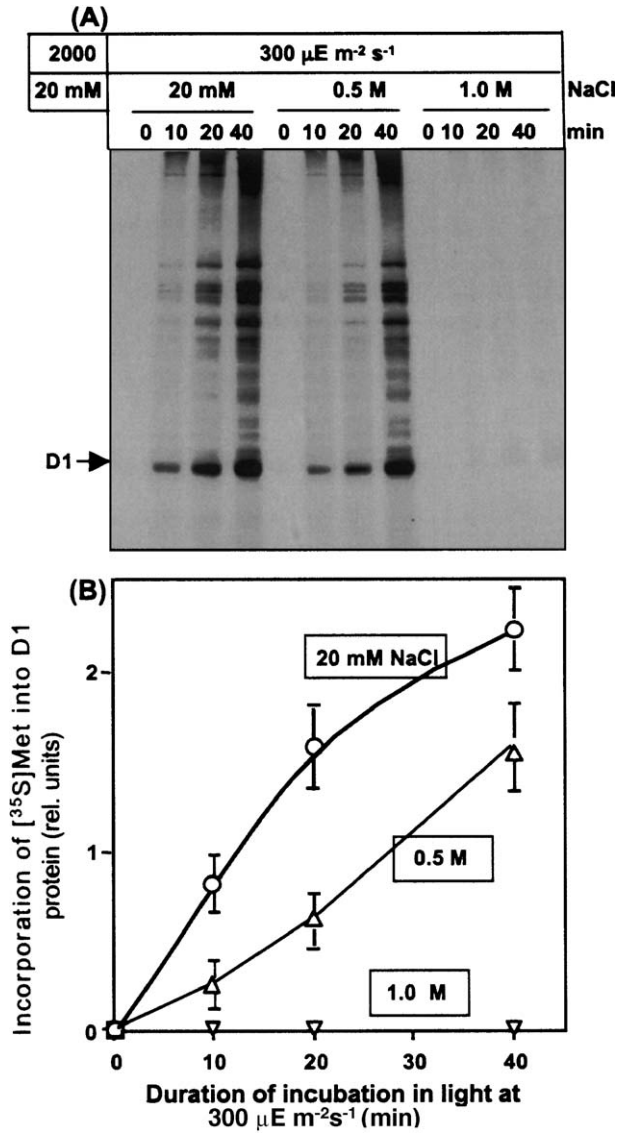

Fig. 6. Effects of NaCl on the synthesis of proteins de novo in *Synechocystis*, as monitored in terms of the incorporation of [^{35}S]Met into proteins of thylakoid membranes under recovery conditions. Cells were incubated, in the absence of lincomycin, in light at 2000 μE m^{-2} s^{-1} to induce 90% inactivation of PSII. Then cells were incubated in light at 300 μE m^{-2} s^{-1} with 10 nM [^{35}S]Met (>1000 Ci $mmol^{-1}$) in the presence of 0.5 or 1.0 M NaCl or in its absence. At designated times, a portion of each suspension of cells was withdrawn for preparation of thylakoid membranes. Proteins from thylakoid membranes were analyzed by polyacrylamide gel electrophoresis as described in Materials and methods. Proteins from thylakoid membranes corresponding to 0.8 μg of Chl were applied to each lane. (A) Patterns of radiolabeled proteins. The arrow indicates the position of D1. The results shown are representative of the results of four independent experiments, each of which gave similar results. (B) The time course of incorporation of [^{35}S]Met into D1 protein. ○, No addition; △ and ▽, in the presence of 0.5 M and 1.0 M NaCl, respectively. Each point and bar represent the average ± S.E. of results from four independent experiments. Other details are the same as described in the legends to Figs. 1 and 3.

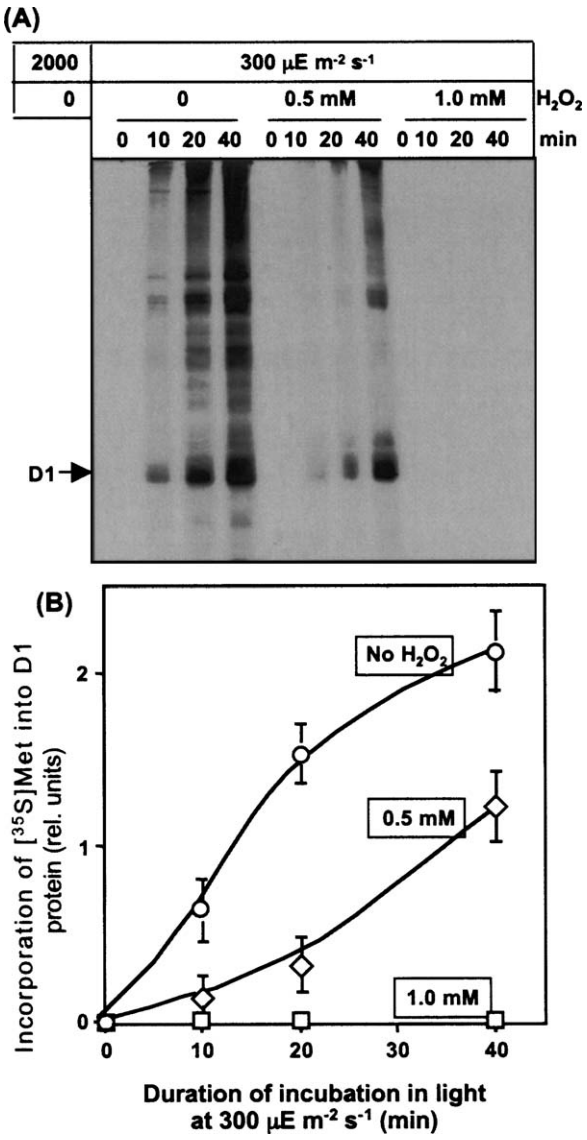

Fig. 7. Effects of H_2O_2 on the synthesis of proteins de novo in *Synechocystis* in the presence of H_2O_2 at various concentrations, as monitored in terms of the incorporation of [^{35}S]Met into proteins of thylakoid membranes under recovery conditions. Cells were incubated, in the absence of lincomycin, in light at 2000 μE m^{-2} s^{-1} to induce 90% inactivation of PSII. Then cells were incubated in light at 300 μE m^{-2} s^{-1} with 10 nM [^{35}S]Met (>1000 Ci $mmol^{-1}$) in the presence of 0.5 mM or 1.0 mM H_2O_2 or in its absence. At designated times, a portion of each suspension of cells was withdrawn for preparation of thylakoid membranes. Proteins from thylakoid membranes were analyzed by polyacrylamide gel electrophoresis as described in Materials and methods. Proteins from thylakoid membranes corresponding to 0.8 μg of Chl were applied to each lane. (A) Patterns of radiolabeled proteins. The arrow indicates the position of D1. The results shown are representative of the results of five independent experiments, each of which gave similar results. (B) The time course of incorporation of [^{35}S]Met into D1 protein. ○, No addition; ◇ and □ in the presence of 0.5 mM and 1.0 mM H_2O_2, respectively. Each point and bar represent the average ± S.E. of results from five independent experiments. Other details are the same as described in the legends to Figs. 1, 3 and 6).

shown in Fig. 8A, the rate of protein synthesis decreased as the temperature of incubation was lowered. At 10 °C, no protein synthesis was detected. Fig. 8B shows the time courses of the incorporation of [^{35}S]Met into the D1 protein at four temperatures. The rate of incorporation of the radioactive amino acid into D1 at 25 °C was 50% of the control rate at 34 °C and the rate at 18 °C was about 15% of the control rate. At 10 °C, no incorporation of radioactivity into D1 was detected.

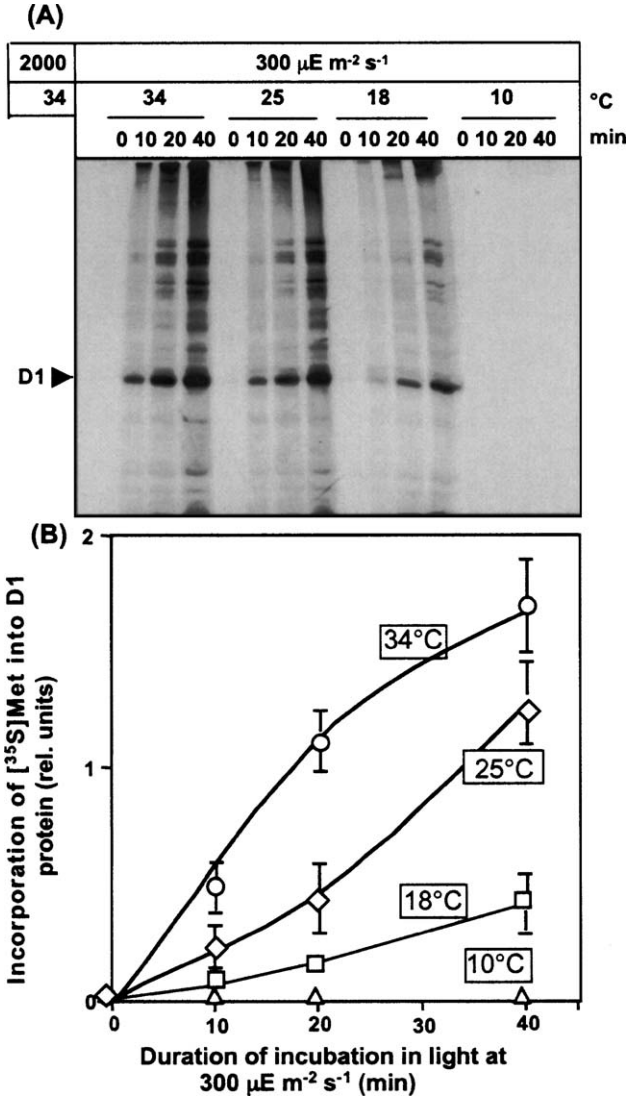

Fig. 8. Effects of various temperatures on the synthesis of the D1 protein de novo in *Synechocystis*, as monitored in terms of the incorporation of [^{35}S]Met into proteins of thylakoid membranes. Cells were incubated, in the absence of lincomycin, in light at 2000 μE m^{-2} s^{-1} at 34 °C for 110 min to induce 90% inactivation of PSII. Then cells were incubated in light at 300 μE m^{-2} s^{-1} at 34, 25, 18 and 10 °C with 10 nM [^{35}S]Met (>1000 Ci $mmol^{-1}$). At designated times, a portion of each suspension of cells was withdrawn for preparation of thylakoid membranes. Proteins from thylakoid membranes were analyzed by polyacrylamide gel electrophoresis as described in Materials and methods. Proteins from thylakoid membranes corresponding to 0.8 μg of Chl were applied to each lane. (A) Patterns of radiolabeled proteins. The arrow indicates the position of D1. The results shown are representative of the results of five independent experiments, each of which gave similar results. (B) The time course of incorporation of [^{35}S]Met into D1 protein. ○, ◇, □ and △, Incorporation at 34 °C, 25 °C, 18 °C and 10 °C, respectively. Each point and bar represent the average ± S.E. of results from five independent experiments. Other details are the same as described in the legends to Figs. 1, 3, 6 and 7).

4. Discussion

In the present study, we determined the rate of photodamage to PSII and the rate of repair of PSII separately by selecting suitable light conditions and by using an inhibitor of protein synthesis. Our results demonstrated clearly that the initial rate of photodamage was proportional to light intensity. This result was in good agreement with results obtained previously with leaves of higher plants, in which protein synthesis is inhibited in the presence of lincomycin [39,40].

It has been assumed that oxidative stress damages the photochemical reaction center of PSII [27–30]. However, our findings suggest that oxidative stress due to H_2O_2 scarcely induces any damage and barely accelerates photodamage to PSII. It has also been assumed that salt stress and low-temperature stress accelerate photodamage to PSII [21,41,42]. However, our findings suggest that salt stress and low-temperature stress do not affect the process by which photodamage occurs.

The linear relationship that we observed between the rate of photodamage and the intensity of light, as well as the absence of any obvious effects of the environmental stresses that we examined, namely, oxidative stress, salt stress and low-temperature stress, on the photodamage to PSII, suggests that photodamage to PSII might occur by simply as a result of the photodynamic action of light. Tyystjärvi et al. [43] proposed that absorption of light by the manganese ions in the oxygen-evolving complex might be the primary event in photodamage to PSII. Our findings may be explained by this hypothesis.

The most interesting observation in the present study was that the repair process depended on light intensity, with the initial rate of repair reaching a half-maximum value at 70 μE m^{-2} s^{-1} and a maximum at 300 μE m^{-2} s^{-1}. At light intensities above 300 μE m^{-2} s^{-1}, the initial rate of repair was always the same, namely, 0.1 min^{-1}.

A comparison between the dependence on light intensity of photodamage and that of repair (Fig. 4) indicated that the rates at which these two processes occur are identical at approximately 900 μE m^{-2} s^{-1}. This observation explains why no apparent photodamage was observed at 500 μE m^{-2} s^{-1}, when repair occurred more rapidly than photodamage (Fig. 1A). At 1000 μE m^{-2} s^{-1} (Fig. 1A), when photodamage occurred slightly more rapidly than repair, the extent of photoinactivation corresponded to about 40% of the maximum value. At 2000 μE m^{-2} s^{-1} (Fig. 1A), photodamage occurred much more rapidly than repair and, as a result, PSII activity eventually disappeared completely (Fig. 1A).

In our previous studies, we determined that salt stress [25] and oxidative stress [24] inhibit the repair of photodamaged PSII by inhibiting the transcription and translation of *psbA* genes. Our studies have also shown that low temperature inhibits the repair of PSII [31,32]. Our present study confirmed the inhibitory effects of environmental stress on the repair of PSII and, in addition, it demonstrated very clearly the manner in which such stress affects repair: it decreases the maximum extent of such repair. As a result, PSII becomes more sensitive to photoinactivation under environmental stress, such as salt stress, oxidative stress and low-temperature stress.

Repair of photodamaged PSII requires several steps [3], which include the degradation of damaged D1 protein, the synthesis de novo of D1 protein, and the incorporation of newly synthesized D1 into PSII [44]. In *Synechocystis*, the D1 protein is encoded by a small multigene family that consists of the *psbAI*, *psbAII* and *psbAIII* genes [45]. However, *psbAI* is not expressed at all [46] and *psbAII* and *psbAIII* are almost identical, encoding a single form of D1 [46–48]. Nevertheless, these two genes are differentially expressed: about 95% of *psbA* transcripts originate from *psbAII*, while only 5% originate from *psbAIII*, in cells that are grown under weak or strong light [47,48]. The present study demonstrated clearly that protein synthesis de novo and the synthesis de novo of the D1 protein, in particular, were inhibited under environmental stress conditions. We noticed, moreover, that the extent of inhibition of repair of PSII by these three types of stress (Fig. 5) was very similar to the extent of their inhibition of the synthesis of D1 protein (Figs. 6–8) when their effects were examined at a light intensity of 300 $\mu E\ m^{-2}\ s^{-1}$, the intensity at which the rate of repair of PSII reached a maximum. These findings suggest that the synthesis of proteins de novo and the synthesis de novo of the D1 protein, in particular, might be the rate-limiting step in the repair cycle in *Synechocystis* under stress conditions.

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