Dissection of Photodamage at Low Temperature and Repair in Darkness Suggests the Existence of an Intermediate Form of Photodamaged Photosystem II†

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ABSTRACT: Irradiation of the photosynthetic machinery with strong light induces damage to the photosystem II complex (PSII), and this phenomenon is referred to as photodamage. In an attempt to characterize the mechanism of photodamage to PSII, we examined the events associated with photodamage by monitoring the phenomenon in *Synechocystis* sp. PCC 6803 at a low temperature. After the activity of PSII had been reduced to 10% of the original activity by exposure of *Synechocystis* cells to strong light at 10 °C, recovery was allowed to proceed at 34 °C in darkness. Under these conditions, approximately 50% of the activity of PSII was restored within 60 min. The recovery in darkness did not require protein synthesis, as demonstrated by Western blotting analysis and a radiolabeling experiment with [^{35}S]methionine. We also observed a similar recovery of PSII in darkness in isolated thylakoid membranes. Our findings, together with those of other studies, suggest the presence of an intermediate form of photodamaged PSII that is generated prior to the formation of photodamaged PSII.

Light is essential for photosynthesis, but it can also be detrimental to the photosynthetic machinery. In particular, strong light induces damage to the photosystem II complex (PSII).[1] The main target of this photodamage seems to be the D1 protein of the PSII reaction center (*1*, *2*; for reviews, see refs *3* and *4*). Two specific proteases, namely, endoprotease DegP2 and metalloprotease FtsH, degrade the D1 protein in the photodamaged PSII (*5*−*7*), and coordinately with the degradation of D1, the precursor to D1 (pre-D1) is synthesized and reassembled with the residual components of PSII to generate a functional PSII in terms of charge separation and reduction of Q_A and Q_B quinones. The precursor to D1 is then processed to yield the mature D1 protein for the complete repair of PSII and restoration of the oxygen-evolving activity (*1*, *2*; for a review, see ref *3*).

In comparison to the mechanism responsible for the repair of PSII, the mechanisms responsible for the photodamage to PSII are poorly understood. It remains to be determined whether photodamage is a single-step reaction or involves several steps. If several steps are involved, it should be possible to dissect the process of photodamage and to identify each individual step. We postulated that a low temperature might allow us to dissect the phenomenon of photodamage and to characterize the individual steps, each of which is likely to have a different temperature coefficient.

In the present study we investigated the effects of low temperature on the photodamage to PSII in intact cells of *Synechocystis* sp. PCC 6803 (hereafter, *Synechocystis*) and in thylakoid membranes isolated from such cells. Our results suggest that photodamage to PSII involves two steps, that an intermediate form of photodamaged PSII is generated prior to the formation of stably damaged PSII, and, in addition, that the processing of the precursor to the D1 protein occurs in light at high (physiological) temperature.

MATERIALS AND METHODS

Cyanobacteria and Culture Conditions. Synechocystis sp. PCC 6803 was kindly donated by Dr. J. G. K. Williams of DuPont de Nemours & Co. (Wilmington, DE). Cells were grown photoautotrophically in glass tubes (25 mm i.d. × 200 mm) at 34 °C under constant illumination from incandescent lamps at 70 μE m^{-2} s^{-1} in BG-11 medium (*8*), supplemented with 20 mM HEPES−NaOH (pH 7.5). Cultures were aerated with sterile air that contained 1% CO_2 (*9*).

Conditions for Photodamage and Recovery. Cells at a density of 3 μg/mL chlorophyll (Chl) were used directly for studies of photodamage. They were incubated for various times under standard growth conditions but were exposed to strong light at various temperatures for the induction of photodamage. After the activity of PSII had been reduced to 10% of the original level, photodamaged cells were

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[1] Abbreviations: BQ, 1,4-benzoquinone; Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; PBQ, phenyl-1,4-benzoquinone; PMSF, phenylmethylsulfonyl fluoride.

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transferred to darkness at various temperatures and incubated for 90 min. Finally, they were exposed to weak light to induce repair at 34 °C under the standard growth conditions.

In some experiments, the synthesis of proteins was inhibited by inclusion in the culture medium of 250 μg/mL lincomycin (Sigma Chemical Co., St. Louis, MO), an inhibitor of the initiation of translation. Lincomycin was added to the culture medium 10 min before the start of incubation.

Preparation of Thylakoid Membranes. Thylakoid membranes were isolated from intact cells of *Synechocystis* as described previously (*10*, *11*), with some modifications. A suspension of cells at a Chl concentration of 5 μg/mL was mixed with an equal volume of glass beads (diameter 0.1 mm; BioSpec Products, Inc., Bartlesville, OK), and the cells were disrupted by vortex mixing for 30 s with glass beads, which was repeated a total of three times, with 30 s interruptions, on ice. The homogenate was centrifuged at 5000*g* for 5 min, and the resultant supernatant was centrifuged at 47000*g* for 35 min. The pelleted thylakoid membranes were suspended at a Chl concentration of 200 μg/mL in a solution that contained 50 mM HEPES−NaOH (pH 7.5), 600 mM sucrose, 30 mM $CaCl_2$, 1.0 mM phenylmethylsulfonyl fluoride (PMSF), 1.0 mM benzamidine, 1.0 M glycinebetaine, and 1.0 mM 6-amino-*n*-caproic acid.

Exposure of Thylakoid Membranes to Light. The suspension of thylakoid membranes was diluted with a solution that contained 50 mM HEPES−NaOH (pH 7.5), 600 mM sucrose, 30 mM $CaCl_2$, 2 mM $MgCl_2$, 1.0 mM PMSF, 1.0 mM benzamidine, 1.0 M glycinebetaine, and 1.0 mM 6-amino-*n*-caproic acid to give a Chl concentration of 10 μg/mL. A 20 mL aliquot of this suspension of thylakoid membranes, in a glass tube (15 mm i.d. × 180 mm), was illuminated at 10 °C in a temperature-controlled growth chamber with light at 800 μE m^{-2} s^{-1} to induce photodamage. After the PSII activity had fallen to 10% of the original level, the thylakoid membranes were transferred to darkness at 34 °C in the absence and in the presence of 250 μg/mL lincomycin. After incubation for 90 min, the thylakoid membranes were exposed to light at 70 μE m^{-2} s^{-1}.

Measurement of the Activity of PSII. The activity of PSII in intact cells at a Chl concentration of 3 μg/mL and in isolated thylakoid membranes at a Chl concentration of 10 μg/mL was determined by monitoring oxygen-evolving activity in the presence of 1.0 mM 1,4-benzoquinone (BQ) and 1.0 mM phenyl-1,4-benzoquinone (PBQ), respectively, as the artificial acceptor of electrons, with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, U.K.). Each sample, in a 3 mL cuvette, was illuminated by light from incandescent lamps that had been passed through a red optical filter (R-60; Toshiba, Tokyo, Japan) and an infrared-absorbing filter (HA-50; Hoya Glass, Tokyo, Japan). The intensity of light at the surface of the cuvette was 2000 μE m^{-2} s^{-1}, which was sufficient to saturate the photosynthetic machinery.

Quantitation of Chl Fluorescence. The yield of Chl fluorescence from intact cells was measured with a pulse amplitude modulation fluorometer (PAM-101; Walz, Effeltrich, Germany). The initial fluorescence of Chl (F_0) was determined after excitation with dim light at 650 nm and 10 μE m^{-2} s^{-1}, which was modulated at 600 Hz. The maximum yield of fluorescence (F_m) was determined after the addition of continuous actinic light at 2700 μE m^{-2} s^{-1} (see refs *12* and *13*). Concentrations of Chl were determined as described by Arnon et al. (*14*).

Western Blotting Analysis. Thylakoid membranes were isolated from cells that had been incubated in light as described above. Proteins in isolated thylakoid membranes, equivalent to 0.8 μg of Chl *a*, were separated by SDS−PAGE on a 12.5% polyacrylamide gel that contained 6 M urea. Molecular markers (Kaleidoscope prestained standards; Bio-Rad Laboratories, Hercules, CA) were used for estimations of the molecular masses of D1 and pre-D1 proteins (see refs *15* and *16*). After electrophoresis, the separated proteins were blotted onto a nitrocellulose membrane (Schleicher & Schuell Inc., Keene, NH) in a semidry transfer apparatus (Atto, Tokyo, Japan). Then D1 and pre-D1 proteins were detected immunologically with an ECL Western blotting kit (Amersham International plc, Amersham, England) according to the protocol supplied by the manufacturer. The D1 protein was detected with rabbit antibodies raised against amino acid residues 55−78 in the AB loop of the D1 protein from spinach (*17*, *18*). These antibodies recognized the products (D1) of the *psbAI*, *psbAII*, and *psbAIII* genes since the amino acid sequences of the AB loops are identical in the products of all three of these genes. The pre-D1 protein was detected with antibodies raised in rabbits against an oligopeptide of 16 amino acid residues (SGEQAPVALTAPAVNG) that corresponded to the carboxyl terminus of pre-D1 (the products of the *psbAII* and *psbAIII* genes) from *Synechocystis* (*16*, *19*, *20*). As second antibodies, we used horseradish peroxidase-linked antibodies raised in donkey against rabbit IgG (Amersham International plc). The antibodies against D1 were kindly provided by Prof. K. Satoh (Department of Biology, Okayama University, Okayama, Japan), and the antibodies against pre-D1 were generated in our laboratory. A digital camera system (LAS-1000; Fuji Photo Film Co., Tokyo, Japan) was used to monitor signals from blotted membranes and to quantify the D1 and pre-D1 proteins.

Radioactive Labeling of Proteins in Vivo. We performed two types of labeling experiments. We incubated *Synechocystis* cells at a concentration that corresponded to 3 μg/mL Chl with 10 nM [^{35}S]methionine (>1000 Ci/mmol; Amersham Pharmacia Biotech, Buckinghamshire, U.K.). In one type of experiment, we added [^{35}S]Met before we exposed cells to strong light for induction of photodamage. In the other type of experiment, we added the radiolabel after photodamage had been induced.

The labeling of proteins with [^{35}S]Met was terminated by the addition of nonradioactive methionine to a final concentration of 1.0 mM and immediate cooling of the samples on ice. Cells were collected by centrifugation at 5000*g* for 5 min at 4 °C, and thylakoid membranes were isolated from these cells as described previously (*16*). 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) SDS, 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) SDS and 6 M urea, as described previously (*21*, *22*). Solubilized thylakoid membranes corresponding to 0.8 μg of Chl *a* were loaded in each 5 mm wide lane of the gel. After electrophoresis, the labeled proteins on the gel were visualized by exposure of the dried and fixed gel to X-ray

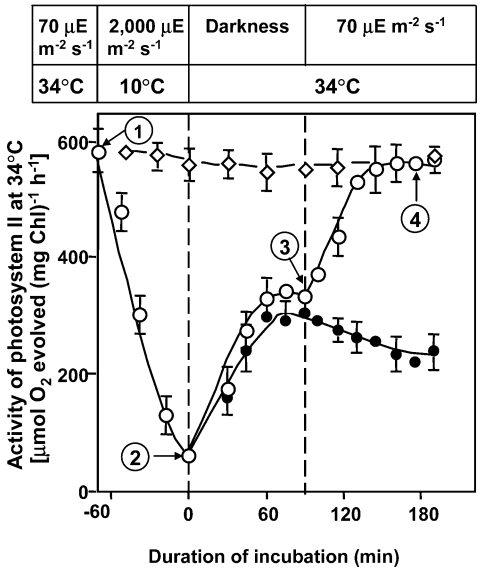

FIGURE 1: Photodamage and dark recovery of PSII in *Synechocystis* cells. Cells were incubated at 10 °C at 2000 μE m^{-2} s^{-1} for 70 min, to induce 90% inactivation of PSII, and then at 34 °C in darkness for 90 min in the presence of 250 μg/mL lincomycin (●) and in its absence (○). Cells were also incubated at 10 °C for 70 min in darkness (◇, control). At designated times, a portion of the cell suspension was withdrawn, and after addition of 1.0 mM BQ to the suspension, PSII activity was examined by monitoring oxygen-evolving activity. Each point and bar represent the average ± SE of results from three independent experiments.

film. Radioactivity of radiolabeled D1 was quantitated with a digital camera system (LAS-1000; Fuji Photo Film Co.).

RESULTS

Recovery from Photodamage in Darkness. Exposure of *Synechocystis* cells to strong white light at 2000 μE m^{-2} s^{-1} at 10 °C rapidly decreased the activity of PSII. Close to 90% of the original activity disappeared within 70 min. When these cells were transferred to the standard growth conditions (light at 70 μE m^{-2} s^{-1}, 34 °C), the PSII activity resumed rapidly, and full recovery from photodamage occurred within 120 min (data not shown). However, when cells that had been similarly photodamaged were transferred to 34 °C in total darkness, a significant recovery from photodamage was also apparent (Figure 1). Recovery in darkness was, however, not complete, but approached 50% of the original level within 60 min. Prolonged incubation did not result in any further recovery of PSII.

No changes in pigment content occurred during photodamage and subsequent recovery in darkness (data not shown). Moreover, addition of 5 mM glucose during the dark period did not result in any significant increase in the extent of recovery of PSII (data not shown), suggesting that the recovery process in darkness might not require a source of chemical energy. When photodamaged cells were transferred to 34 °C with illumination by weak light at 70 μE m^{-2} s^{-1}, after they had been incubated in darkness for 90 min, PSII activity returned to the original level within 60 min (Figure 1).

Figure 1 also shows the effects of lincomycin, an inhibitor of protein synthesis, on the recovery of PSII. The recovery of PSII activity in darkness was unaffected by lincomycin. However, lincomycin did prevent repair in weak light (70 μE m^{-2} s^{-1}). These observations indicated that the recovery of photodamaged PSII in darkness did not require protein synthesis. Thus, this aspect of the recovery process was distinct from light-dependent repair that involves the synthesis of proteins de novo and reassembly of PSII.

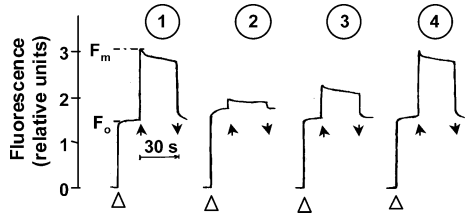

FIGURE 2: Changes in the variable fluorescence of Chl *a* during photodamage and the subsequent repair of PSII. Cells were incubated as described in the Figure 1 caption. A portion of the cell suspension was withdrawn at the times indicated by numbers in circles in Figure 1. Open triangles indicate the time when the measuring light, used to excite Chl (λ = 650 nm, 10 μE m^{-2} s^{-1}), was turned on; upward and downward arrows indicate when actinic light (λ > 600 nm, 2700 μE m^{-2} s^{-1}) was turned on and off, respectively. The concentration of Chl was 3 μg/mL, and the experiment was performed at 34 °C.

Figure 1 also shows that exposure of cells to 10 °C for 70 min in darkness and subsequently to 34 °C for 90 min also in darkness had no effect on the activity of PSII, suggesting that the inactivation of PSII by strong light at 10 °C was due to the combined effect of light and low temperature.

We examined the repair of PSII in darkness after photodamage by fluorometry. Figure 2 shows that, before photodamage, actinic illumination increased the yield of Chl *a* fluorescence (trace 1). The yield was much smaller after PSII had been photodamaged at 10 °C by strong light at 2000 μE m^{-2} s^{-1} (Figure 2, trace 2). When cells had been incubated at 34 °C in darkness after photodamage, the yield recovered to half of the control yield (Figure 2, compare traces 3 and 1). Full recovery was achieved after cells had been incubated for a further 90 min at 34 °C in weak light at 70 μE m^{-2} s^{-1} (Figure 2, trace 4). These results suggested that photodamage and subsequent repair, as shown in Figure 1, were events that occurred in the photochemical reaction center of PSII.

Dependence on Temperature of Recovery in Darkness. We examined the effects of the temperature during the incubation in darkness on the recovery process. Figure 3 shows that recovery during the incubation in darkness occurred at high temperatures, such as 25 and 34 °C, but not at low temperatures, such as 0 and 10 °C, suggesting that recovery in darkness required processes that were operative only at higher temperatures.

We next examined the effects of the incubation temperature during photodamage on subsequent recovery in darkness at 34 °C. Figure 4 shows that recovery in darkness depended on a low temperature, such as 0 or 10 °C, during photodamage. No recovery of PSII in darkness was detectable when PSII had been photodamaged at temperatures above 20 °C. These findings indicated that recovery of PSII in darkness, without any synthesis of D1 protein de novo, was detectable only when *Synechocystis* cells had been exposed to strong light at a low temperature and then incubated in darkness at a high temperature.

Figures 3 and 4 also show that, after the cells were transferred to the conditions of 34 °C and 70 μE m^{-2} s^{-1}, they completely recovered the PSII activity irrespective of

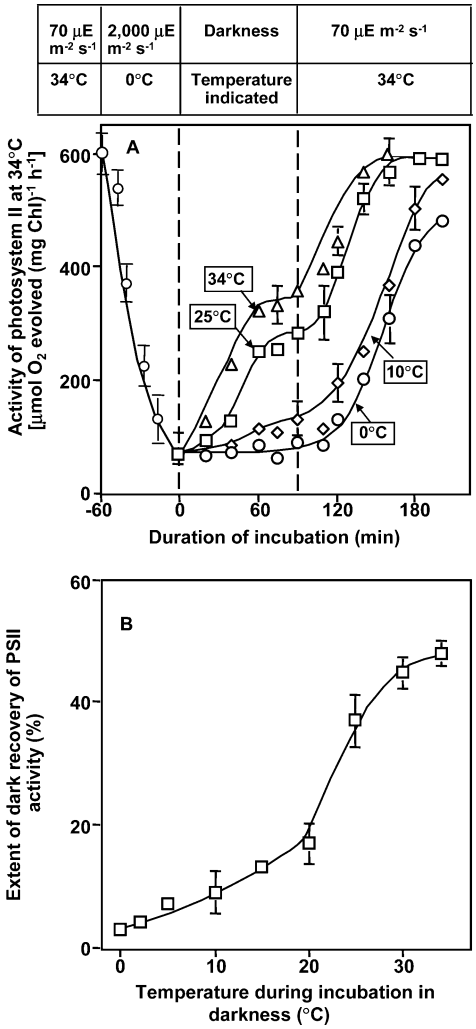

FIGURE 3: Effects of temperature on the dark recovery of PSII activity. Cells were incubated at 0 °C at 2000 μE m^{-2} s^{-1} for 60 min to induce 90% inactivation of PSII, and then they were incubated in darkness at various temperatures for 90 min, after which incubation was continued at 70 μE m^{-2} s^{-1} at 34 °C. All other experimental conditions were the same as indicated in the Figure 1 caption. (A) Time course of the recovery in darkness. (B) Dependence on temperature of the recovery in darkness, which is presented as a percentage of the total recovery. Each point and bar represent the average ± SE of results from four independent experiments.

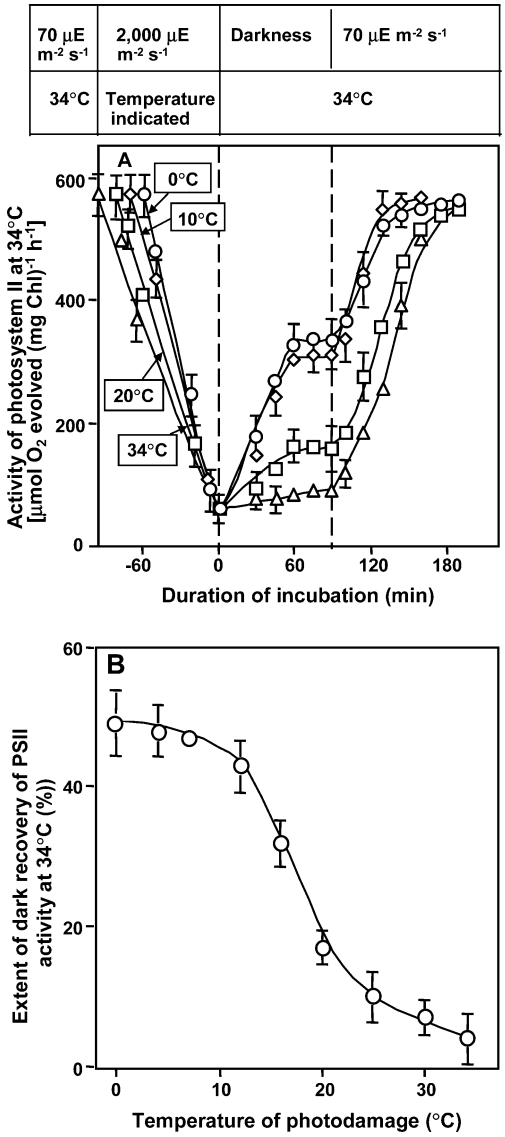

FIGURE 4: Effects of temperature during photodamage on the dark recovery of PSII activity at 34 °C. Cells were incubated at various temperatures at 2000 μE m^{-2} s^{-1} for several minutes to induce 90% inactivation of PSII, and then they were incubated in darkness at 34 °C for 90 min, after which incubation was continued at 70 μE m^{-2} s^{-1} at 34 °C. All other experimental conditions were the same as described in the Figure 1 caption. (A) Time course of photodamage and recovery of PSII. (B) Dependence on temperature of the recovery in darkness at 34 °C, which is presented as a percentage of the total recovery. Each point and bar represent the average ± SE of results from five independent experiments.

the temperature of photodamage and of the subsequent incubation in darkness. These findings suggested that the ability of the cells to repair PSII was unaffected by the temperature during the previous incubation.

Western Blotting Analysis of the D1 Protein and Its Precursor. Figure 5 shows the results of Western blotting analysis of D1 and pre-D1 at the time points indicated in Figure 1. The level of D1 protein remained constant during photodamage and recovery in darkness (lanes 2 and 3, respectively), suggesting that, at a low temperature such as 10 °C, the D1 protein in photodamaged PSII was not degraded.

The level of pre-D1 increased during photodamage at 10 °C and remained at a constant level during incubation at 34 °C in darkness for 90 min (Figure 5, lanes 2 and 3). However, the level of pre-D1 decreased during the subsequent incubation in weak light at 70 μE m^{-2} s^{-1} at 34 °C (Figure 5, lane 4), suggesting that pre-D1 might be converted to D1 during incubation under these conditions. These observations indicated that pre-D1, which was synthesized in strong light at 10 °C, was not converted to D1 at 34 °C in darkness and, moreover, that light was necessary for the conversion of pre-D1 to D1. Therefore, it appeared that the conversion of pre-D1 to D1 might not be necessary for the recovery of PSII from photodamage during incubation at 34 °C in darkness.

Examination of Protein Synthesis by Radioactive Labeling. We examined protein synthesis during photodamage and repair by monitoring the incorporation of [^{35}S]Met into the proteins of thylakoid membranes (Figure 6). When we added [^{35}S]Met prior to the start of incubation at 10 °C in light at 2000 μE m^{-2} s^{-1}, a limited amount of radioactivity was detected in pre-D1, but in no other protein, after incubation for 70 min (Figure 6A, lane 2). The radioactivity of pre-D1

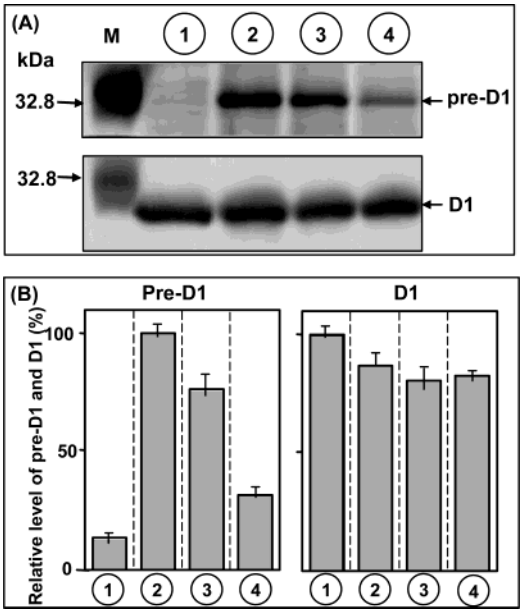

FIGURE 5: Western blotting analysis of pre-D1 and D1 proteins during photodamage and subsequent repair of PSII. Cells were incubated as described in the Figure 1 caption. A portion of the suspension of cells was withdrawn at the times indicated by numbers in circles in Figure 1, and then thylakoid membranes were isolated from the cells as described in the Materials and Methods. Two preparations of antibodies, specific for D1 and pre-D1, respectively, were used for immunodetection of these proteins. Samples equivalent to 0.8 μg of Chl *a* were loaded in each well. (A) Pre-D1 and D1 proteins after gel electrophoresis. Identical results were obtained in four independent experiments. (B) Quantitation of the results in (A). Each point and bar represent the average ± SE of results from four independent experiments.

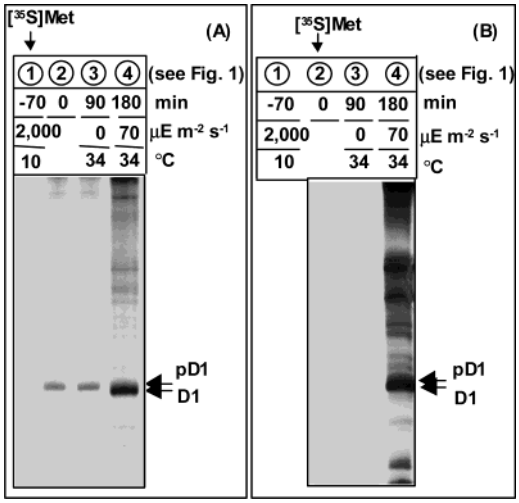

FIGURE 6: Synthesis of pre-D1 and D1 proteins de novo in *Synechocystis*, as monitored in terms of the incorporation of radioactive [^{35}S]methionine into proteins of thylakoid membranes. Cells were incubated as described in the Figure 1 caption. A portion of the cell suspension was withdrawn at the times indicated by numbers in circles in Figure 1, and then thylakoid membranes were isolated from the cells. The proteins from thylakoid membranes were analyzed by PAGE as described in the Materials and Methods. Proteins from thylakoid membranes corresponding to 0.8 μg of Chl were loaded in each lane. (A) A culture of *Synechocystis* cells at a concentration that corresponded to 3 μg/mL Chl *a* was supplemented with [^{35}S]methionine prior to incubation at 10 °C in light at 2000 μE m^{-2} s^{-1}. (B) A culture of *Synechocystis* cells was supplemented with [^{35}S]methionine after incubation for 70 min at 10 °C in light at 2000 μE m^{-2} s^{-1}, prior to incubation in darkness at 34 °C. The results shown are representative of the results of three independent experiments, each of which gave similar results.

remained almost unchanged during incubation in darkness at 34 °C (Figure 6A, lane 3). Higher levels of radioactivity were incorporated into pre-D1 and D1 during subsequent incubation in weak light at 34 °C (Figure 6A, lane 4). Compared to the synthesis of D1 and pre-D1, the synthesis of other proteins at all times during this experiment was insignificant.

When we added [^{35}S]Met after the photodamaging incubation in light at 2000 μE m^{-2} s^{-1} and before the incubation for induction of recovery in darkness, we detected no incorporation of radioactivity into pre-D1 and D1 during the dark incubation at 34 °C (Figure 6B, lane 3), even though the activity of PSII increased from 10% to 50% of the original level. Incorporation of radioactivity into pre-D1 and D1, as well as into other proteins, was detected only during the incubation in light (Figure 6B, lane 4), when the activity of PSII increased from 50% to 100% of the original level (see Figure 1). These results suggested that, at a low temperature such as 10 °C, pre-D1 was synthesized at a low level but was not processed. These observations also indicated that no pre-D1 was synthesized during the incubation in darkness at 34 °C, when the activity of PSII returned to 50% of the original level.

Recovery from Photodamage in Darkness in Isolated Thylakoid Membranes. To determine whether the recovery of PSII activity in darkness occurred only in vivo or could also be detected in vitro, we examined the dark recovery of PSII activity in isolated thylakoid membranes. Figure 7 shows the dark recovery of PSII activity at 34 °C in this system, demonstrating that recovery occurred in isolated thylakoid membranes, even though the extent of recovery was slightly smaller than that in intact cells. No further recovery of PSII activity was detected during a subsequent incubation in weak light. The presence of lincomycin had no effect on the activity of PSII in isolated thylakoid membranes in darkness or in weak light.

DISCUSSION

Our experiments demonstrated that significant recovery of PSII activity occurs at 34 °C in darkness after photodamage to PSII of *Synechocystis* has been induced by strong light at a low temperature, such as 0 or 10 °C. The discovery of such repair of PSII in darkness is novel because the recovery process does not require protein synthesis and the extent of recovery is very significant. In a previous study, Constant et al. (*23*) observed the recovery of PSII activity in darkness after *Synechocystis* cells had been incubated at 22 °C in light at 4000 μE m^{-2} s^{-1} for 30 min and then in darkness for 120 min. However, the extent of the recovery in darkness that they observed was very limited (only 8%, from 32% to 40% of the original activity), and recovery was completely blocked by lincomycin. These observations suggest that protein synthesis might have been involved in this minimal recovery in darkness. Kirilovsky et al. (*24*) observed, in *Synechocystis* cells, the recovery in darkness of PSII activity, which was measured by means of variable fluorescence. This recovery occurred in the presence of chloramphenicol, but its extent was only 10% of the total activity. Ohad et al. (*25*, *26*) observed, during incubation of *Chlamydomonas* cells in darkness after exposure to strong

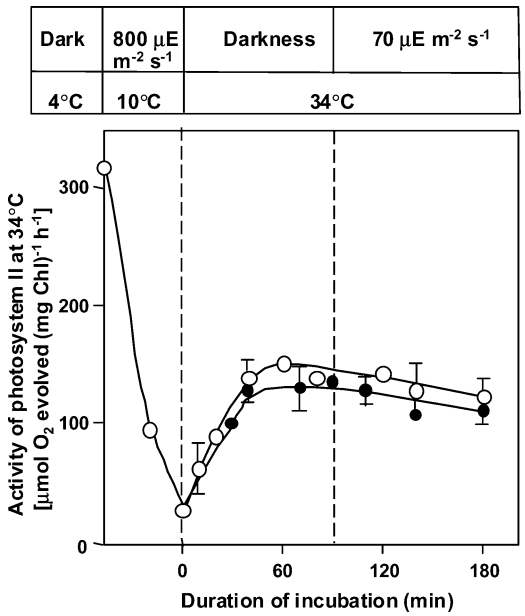

FIGURE 7: Photodamage and recovery of photosystem II activity in isolated thylakoid membranes. Thylakoid membranes were incubated at 10 °C in light at 800 μE m^{-2} s^{-1} for 47 min to induce 90% inactivation of PSII, and then at 34 °C in darkness for 90 min in the presence of 250 μg/mL lincomycin (●) and in its absence (○). Then incubation was continued at 34 °C in light at 70 μE m^{-2} s^{-1}. At designated times, a portion of the suspension was withdrawn, and after addition of 1.0 mM PBQ to the suspension, PSII activity was examined by monitoring oxygen-evolving activity. Each point and bar represent the average ± SE of results from four independent experiments.

light, some extent of the shift in temperature and the recovery of intensity of the B-band in the temperature profile of thermoluminescence. These changes occurred in the presence of spectinomycin, an inhibitor of protein synthesis in the chloroplast. However, this dark recovery does not exceed 10−15% of the total activity of PSII.

On the other hand, Hundal et al. (*27*) observed recovery of the PSII activity in darkness in thylakoid membranes from spinach after the thylakoid membranes were exposed to strong light under anaerobic conditions. Since the recovery in isolated thylakoid membranes does not depend on the protein synthesis, there may exist a mechanism commonly underlying the recovery in darkness after photodamage at low temperature and under anaerobic conditions. However, we need a further study to clarify this question.

The results of our analysis of the recovery of PSII activity in darkness can be summarized as follows: (1) The recovery was observed when photodamage was induced between 0 and 10 °C (Figure 4). (2) Recovery in darkness was induced at 30−35 °C (Figure 3). (3) The extent of recovery in darkness was 50% of the total possible recovery (Figures 3 and 4), with the remaining recovery being light-dependent and inhibited by lincomycin, an inhibitor of protein synthesis (Figure 1). (4) Recovery in darkness involved events in the photochemical reaction center of PSII (Figure 2). (5) Recovery in darkness was unaffected by lincomycin, and thus, protein synthesis was probably not involved in this repair process (Figure 1). (6) During the recovery in darkness, no D1 protein was synthesized (Figures 5 and 6). (7) Recovery in darkness was detected in cells and in isolated thylakoid membranes, suggesting that the process can occur

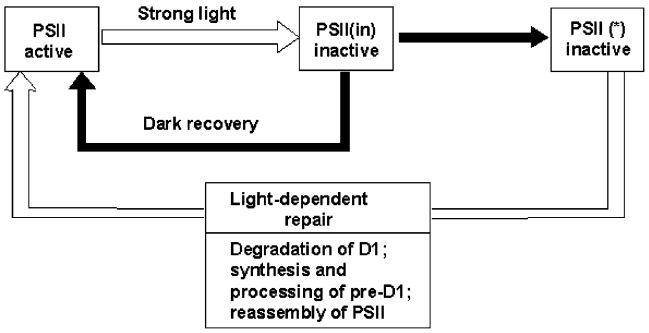

FIGURE 8: A schematic representation of the proposed steps in the photodamage and dark repair of PSII.

in the absence of cellular activities, such as protein synthesis and other cytosolic reactions (Figure 7).

The characteristics of the recovery in darkness cannot be explained by the currently accepted photodamage and repair cycle, and we suggest the hypothetical scheme that is shown in Figure 8. This scheme includes a putative intermediate in the photodamage to PSII, which we designate PSII(in). This intermediate is converted to stably photodamaged PSII(*), which is then repaired to yield PSII(active). Another possible hypothesis is that strong light converts PSII(active) into PSII-(in) at a low quantum yield, regardless of temperature.

All our experimental results can be fully explained by this scheme. Thus, at low temperatures, from 0 to 10 °C, the reactions that convert PSII(in) to PSII(active) and then to PSII(*) do not occur, and as a result, PSII(in) accumulates. When cells are subsequently transferred to 34 °C and darkness, half of the accumulated PSII(in) is converted to PSII(active), such that PSII activity returns to 50% of the original level, while the other half is converted to PSII(*), which is processed via the light-dependent repair cycle. However, when cells are exposed to strong light at a high temperature, such as 34 °C, both reactions are rapid and only PSII(*) accumulates. Thus, we observe light-dependent repair exclusively after cells have been exposed to strong light at a normal temperature, such as 34 °C. This scheme explains all the features of the recovery in darkness, such as the dependence on light and temperature and the lack of requirement for protein synthesis.

The exact nature of the intermediate form of PSII is unknown, but its existence seems not to be in doubt. The recovery from photodamage without protein synthesis might be important under certain physiological conditions, for example, when a low temperature prevents the degradation of D1 and recovery in darkness can be induced at a favorable temperature.

Our dissection of the photodamage and repair cycle using low temperatures produced another piece of important information about the synthesis and processing of pre-D1 (as summarized in Table 1). As shown in Figures 5 and 6A, pre-D1 was synthesized, albeit at an insignificant level, at 10 °C. Thus, it is likely that transcription and translation occurred at 10 °C even though the rates of both were very low. However, it is also very likely that pre-D1 was not processed at this temperature. Even at 34 °C, pre-D1 was processed only very slowly in darkness, and it was only when *Synechocystis* cells were exposed to light at 34 °C that pre-D1 was processed effectively. This scenario was confirmed by results of the [^{35}S]Met-labeling experiments (Figure 6).

Table 1: Effects of Light and Temperature on the Synthesis and Processing of Pre-D1

conditions		pre-D1	
temp (°C)	light (μE m^{-2} s^{-1})	synthesis	processing
10	2000	slow (Figures 5 and 6A)	none (Figure 5)
34	0	none (Figure 6B)	slow (Figure 5)
34	70	fast (Figure 6)	fast (Figures 5 and 6B)

Thus, it was the processing of pre-D1 that was the step that was most sensitive to a decrease in temperature among the various processes involved in the synthesis of mature D1 protein, and moreover, the synthesis and processing of pre-D1 required light. It is likely that the assembly of pre-D1 into PSII, which occurs before the processing of pre-D1 (*3*, *28*, *29*), might be the step that is sensitive to low temperature. This hypothesis is supported by the fact that the D1 protein in photodamaged PSII was not degraded at 10 °C or at 34 °C in darkness (Figure 5).

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