Singlet Oxygen Inhibits the Repair of Photosystem II by Suppressing the Translation Elongation of the D1 Protein in *Synechocystis* sp. PCC 6803†

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ABSTRACT: Singlet oxygen, generated during photosynthesis, is a strong oxidant that can, potentially, damage various molecules of biological importance. We investigated the effects in vivo of singlet oxygen on the photodamage to photosystem II (PSII) in the cyanobacterium *Synechocystis* sp. PCC 6803. Increases in intracellular concentrations of singlet oxygen, caused by the presence of photosensitizers, such as rose bengal and ethyl eosin, stimulated the apparent photodamage to PSII. However, actual photodamage to PSII, as assessed in the presence of chloramphenicol, was unaffected by the production of singlet oxygen. These observations suggest that singlet oxygen produced by added photosensitizers acts by inhibiting the repair of photodamaged PSII. Labeling of proteins in vivo revealed that singlet oxygen inhibited the synthesis of proteins de novo and, in particular, the synthesis of the D1 protein. Northern blotting analysis indicated that the accumulation of *psbA* mRNAs, which encode the D1 protein, was unaffected by the production of singlet oxygen. Subcellular localization of polysomes with bound *psbA* mRNAs suggested that the primary target of singlet oxygen might be the elongation step of translation.

Oxygen is essential for the viability of most organisms, but it is also potentially toxic to all forms of life. In its ground state, oxygen is a triplet molecule that is generally unreactive as a result of spin restriction. The activation of the ground-state oxygen by various reactions overcomes the spin restriction with resultant formation of reactive oxygen species (ROS). Reduction of oxygen leads to the formation of the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (·OH), while electronic excitation leads to the formation of singlet-state oxygen (singlet oxygen; $^1O_2$). These various ROS can damage cellular components, such as proteins, lipids, and nucleic acids (*1*).

In photosynthetic organisms, the major source of ROS is the photosynthetic machinery in thylakoid membranes, where O_2^-, H_2O_2, and ·OH are generated as a result of the photosynthetic transport of electrons and $^1O_2$ is generated by the transfer of excitation energy from photoexcited pigments (*2*). The generation of ROS is promoted when the photosynthetic machinery absorbs excess light or the availability of CO_2 or NADP is limited (*3*, *4*).

Absorption of excess light also results in a decrease in photosynthetic capacity. Although full details of mechanisms responsible for the light-induced inactivation (photoinhibition) of the photosynthetic machinery remain unclear, the primary target of photodamage is known to be photosystem II (PSII),[1] a complex of proteins and pigments that is the site of the photochemical reaction and the subsequent transport of electrons from water to plastoquinone. Photodamage to PSII is considered to be caused by damage to the D1 protein, which forms a heterodimer with the D2 protein in the reaction center of PSII, and by the subsequent rapid degradation of the D1 protein (*5*−*7*).

Many hypothetical mechanisms have been proposed for the photodamage to PSII, and some of them suggest that ROS and, in particular, $^1O_2$ are involved in photodamage. According to the "acceptor-side" hypothesis, the double reduction of Q_A, a semiquinone acceptor, due to excess light facilitates the formation of the triplet state of the reaction center chlorophyll (Chl), which transfers excitation energy to oxygen molecules, and the $^1O_2$ that is produced damages the D1 protein (*8*). By contrast, the "low-light" hypothesis suggests that, under low-intensity light, charge recombination

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[1] Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; PSII, photosystem II; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TEMP, 2,2,6,6-tetramethylpiperidine; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl.

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between Q_A^- or Q_B^-, another semiquinone acceptor, and the oxidized $S_{2,3}$ states of the donor side of PSII produces triplet Chl, which is responsible for the formation of $^1O_2$ (*9*). The generation of $^1O_2$ can be detected in PSII complexes, thylakoid membranes, and plant leaves that are illuminated (*10*−*12*). Exposure of thylakoid membranes to $^1O_2$ results in the selective and specific cleavage of the D1 protein (*13*). Other ROS, such as H_2O_2 and O_2^-, are generated in illuminated PSII (*14*, *15*), and they also induce specific cleavage of the D1 protein in vitro (*16*). However, since the cited studies were conducted in vitro using isolated thylakoid membranes or PSII complexes that lacked the repair system, the roles of ROS in photodamage in vivo remain to be fully clarified.

In living photosynthetic cells, a system exists for the repair of photodamaged PSII (*6*, *7*). The damaged D1 protein is replaced by a newly synthesized precursor to the D1 protein, which is encoded by the *psbA* genes (*17*, *18*). The carboxy-terminal region of the precursor protein is removed by a specific protease (*19*, *20*), and PSII is reactivated.

We demonstrated recently that, in vivo, H_2O_2 and O_2^- act primarily by inhibiting the repair of photodamaged PSII and not by accelerating the photodamage to PSII directly (*21*). Our investigations of targets of inhibition revealed that such ROS have a negative effect primarily on the synthesis of the D1 protein de novo at the translation step and, thus, inhibit the repair of PSII.

In the present study, we examined whether $^1O_2$, as distinct from H_2O_2 and O_2^-, acts primarily by damaging PSII directly or by inhibiting the repair of photodamage to PSII in vivo in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*). We found that increases in intracellular concentrations of $^1O_2$ inhibited the repair of PSII. Labeling of proteins in vivo and Northern and Western blotting demonstrated that $^1O_2$ acts primarily to inhibit the synthesis of the D1 protein de novo at the elongation step, as does H_2O_2.

EXPERIMENTAL PROCEDURES

Organism and Culture Conditions. Wild-type cells of *Synechocystis* were grown photoautotrophically at 34 °C in BG-11 medium under light at 70 μmol of photons m^{-2} s^{-1} with aeration by sterile air that contained 1% CO_2 (*22*). Cells at a density of 5 ± 0.5 μg mL^{-1} Chl were used for studies of photodamage and repair.

Chemicals and Enzymes. Rose bengal (Wako Pure Chemical Industries, Osaka, Japan), methylene blue (Nakalai Tesque, Kyoto, Japan), eosin yellow (Nakalai Tesque), ethyl eosin (Waldeck GmbH & Co., Münster, Germany), protoporphyrin (Tokyo Chemical Industry, Tokyo, Japan), 2,2,6,6-tetramethylpiperidine (TEMP; Wako Pure Chemical Industries), Na_2Mn-EDTA (Katayama Chemical Inc., Osaka, Japan), glucose oxidase (Oriental Yeast Co. Ltd., Tokyo, Japan), and catalase (Sigma-Aldrich, Inc., St. Louis, MO) were used.

Conditions for Photodamage and Repair and for Production of $^1O_2$. Cells were incubated at 30 °C in light at 1.5 mmol of photons m^{-2} s^{-1} (unless otherwise noted) to induce photodamage to PSII or in light at 70 μmol of photons m^{-2} s^{-1} to induce the repair of photodamaged PSII. Chloramphenicol was added at a final concentration of 200 μg mL^{-1} for the examination of actual photodamage. For studies of photodamage, $^1O_2$ was generated by incubation of cells in light at 1.5 mmol of photons m^{-2} s^{-1} in the presence of the photosensitizers, rose bengal, methylene blue, eosin yellow, ethyl eosin, and protoporphyrin, at designated concentrations. For studies of the effects of $^1O_2$ on repair, cells were incubated in the presence of 10 μM rose bengal in weak light at 70 μmol of photons m^{-2} s^{-1} or in green light at 250 μmol of photons m^{-2} s^{-1}, which was provided via an interference filter with maximum transmittance at 550 nm and a half-width of 10 nm (Edmund Industrial Optics, Barrington, NJ). Rose bengal absorbs light maximally at this wavelength, but Chls and phycobilisomes absorb green light much less effectively.

Examination of the Relationship between Photodamage and Light Intensity. Cells were incubated at 30 °C in light at 250, 500, 1000, 1250, 1500, or 2000 μE m^{-2} s^{-1}, which was generated by passing light through neutral density filters (PC-S380; Hoya Glass, Tokyo, Japan). The synthesis of proteins was blocked by the presence of 200 μg mL^{-1} chloramphenicol. Anaerobic conditions during photodamage were achieved by incubation of cells in the presence of 10 mM glucose, 50 units mL^{-1} glucose oxidase, and 1000 units mL^{-1} catalase, for removal of intracellular oxygen, in addition to 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which prevents the evolution of oxygen. After incubation for designated times, 5 mL of the suspension of cells was withdrawn. Cells were washed twice with fresh BG-11 medium to remove DCMU and then suspended in BG-11 medium at a density of 5 μg mL^{-1} Chl for measurement of the activity of PSII. Initial rates of photodamage to PSII were determined by fitting the photodamage to a first-order reaction curve.

Measurements of Photosynthetic Activity. The activity of PSII was measured at 30 °C by monitoring the evolution of oxygen with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, U.K.) in the presence of 1.0 mM 1,4-benzoquinone as the acceptor of electrons, as described previously (*22*). Concentrations of Chl were determined as described by Arnon et al. (*23*).

Detection of $^1O_2$. The production of $^1O_2$ in suspensions of cells was monitored in terms of the electron paramagnetic resonance (EPR) spectrum of a stable nitroxide radical, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), which was produced by the reaction of 2,2,6,6-tetramethylpiperidine (TEMP) with $^1O_2$ (*11*, *24*, *25*). Prior to measurements, cells were incubated in light at 1.5 mmol m^{-2} s^{-1} in the presence of 10 mM TEMP and a photosensitizer, rose bengal or ethyl eosin, at designated concentrations. At designated times, 40 μL aliquots were withdrawn and supplemented with 50 mM Na_2Mn-EDTA, which quenches the EPR signals from TEMPO that is located outside cells (*26*). The resulting mixture was immediately enclosed in a sealed glass capillary tube (i.d. 0.02 cm). The X-band spectra were recorded at 25 °C with an EPR spectrometer (ER 200-D; Bruker, Karlsruhe, Germany) at a microwave frequency of 9.81 GHz, a microwave power of 10 mW, and a modulation frequency of 100 kHz.

Labeling of Proteins in Vivo. For pulse labeling, cell cultures were supplemented with 10 nM [^{35}S]methionine (>1000 Ci $mmol^{-1}$; Amersham Biosciences, Piscataway, NJ) and then incubated at 30 °C for designated times in light at

1.5 mmol of photons m^{-2} s^{-1} in the presence of a photosensitizer or in its absence. For pulse−chase, cells were pulse-labeled for 15 min in the above conditions and then supplemented with 4 mM nonradioactive methionine. Labeled cells were incubated at 30 °C for designated times in light at 1.5 mmol of photons m^{-2} s^{-1} in the presence of a photosensitizer or in its absence. Thylakoid membranes were isolated as described previously (*27*). Proteins in thylakoid membranes that corresponded to 1.6 μg of Chl were separated by SDS−PAGE on a 12.5% polyacrylamide gel that contained 6 M urea, and labeled proteins were visualized by autoradiography, as described previously (*21*). Levels of the labeled D1 protein were determined densitometrically.

Western Blotting. Proteins in isolated thylakoid membranes, equivalent to 1 μg of Chl, were separated by SDS−PAGE on a 12.5% polyacrylamide gel that contained 6 M urea. Western blotting was performed using rabbit antibodies raised against a synthetic oligopeptide that corresponded to the AB loop of the D1 protein or the carboxy-terminal extension of pre-D1 from *Synechocystis*, as described previously (*21*).

Northern Blotting. Northern blotting was performed with a 1.0 kb fragment of DNA that contained part of the coding region of the *psbA2* gene as probe, as described previously (*21*).

Analysis of Polysomes. Cytosolic polysomes, membrane-bound polysomes, and polysome-free fractions from *Synechocystis* cells were prepared as described previously (*21*). RNAs were isolated from polysomes and polysome-free fractions as described previously (*28*) and subjected to Northern blotting analysis.

RESULTS

Effects of the $^1O_2$-Generating System on the Acceleration of Photodamage to PSII. The incubation of *Synechocystis* cells for 2 h in light at 1.5 mmol of photons m^{-2} s^{-1} had no significant effect on the activity of PSII (Figure 1A, open circles). However, in the presence of chloramphenicol, an inhibitor of protein synthesis, similar incubation resulted in the inactivation of PSII (Figure 1B, open circles). These observations indicate that, at this light intensity, the rate of photodamage was related to the rate of repair, which requires the synthesis of proteins de novo.

We then examined the actions of $^1O_2$ by modulating its intracellular concentration with rose bengal, a photosensitizer that produces $^1O_2$ under illumination. The presence of rose bengal accelerated the apparent photodamage to PSII in light at 1.5 mmol of photons m^{-2} s^{-1} (Figure 1A, open triangles and squares). However, when repair of PSII was blocked by chloramphenicol, the presence of rose bengal had no effect on the progress of photodamage (Figure 1B, open triangles and squares). In darkness, the presence of rose bengal had no effect on the activity of PSII.

Table 1 shows effects of various photosensitizers on photodamage to PSII. Not only hydrophilic photosensitizers, such as rose bengal, methylene blue, and eosin yellow, but also hydrophobic photosensitizers, such as ethyl eosin and protoporphyrin, accelerated the apparent photodamage to PSII in light at 1.5 mmol of photons m^{-2} s^{-1}. However, none of these compounds affected the progress of actual photodamage in the presence of chloramphenicol. These hydrophobic photosensitizers were found to accumulate in thylakoid membranes after isolation of the membranes (data not shown). Thus, it appears that the production of $^1O_2$ in thylakoid membranes might not damage PSII directly.

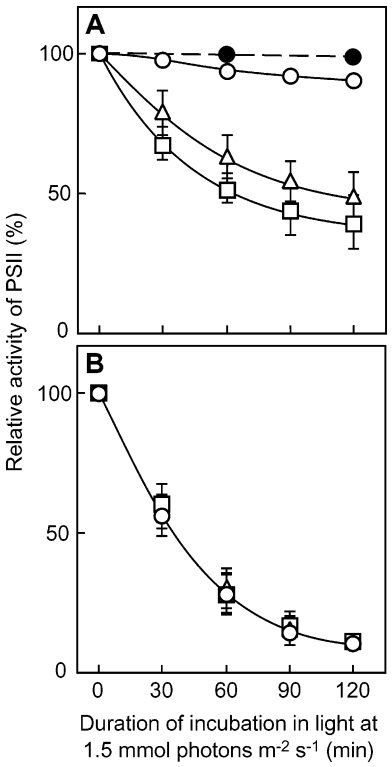

FIGURE 1: Light-induced inactivation of PSII in the presence of rose bengal, which generates $^1O_2$ in *Synechocystis*. Cells were incubated in light at 1.5 mmol of photons m^{-2} s^{-1} at 30 °C with standard aeration (A) in the presence of rose bengal at 2 μM (open triangles) and at 10 μM (open squares) and in its absence (open circles). Closed circles represent inactivation in the presence of 10 μM rose bengal in darkness. (B) The same as (A) but in the presence of 200 μg mL^{-1} chloramphenicol. PSII activity was monitored in terms of the evolution of oxygen in the presence of 1 mM 1,4-benzoquinone as the electron acceptor. The activity taken as 100% was 575 ± 53 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}. Values are means ± SD (bars) of results from four independent experiments.

Table 1: Effects of Various Photosensitizers on the Extent of Apparent Photodamage to PSII in *Synechocystis*[a]

	activity of PSII [μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}]	
photosensitizer	−Cm	+Cm
none	546 ± 17 (97.3)	272 ± 15 (48.5)
hydrophilic		
rose bengal	352 ± 27 (62.7)	263 ± 7 (46.8)
methylene blue	304 ± 53 (54.1)	265 ± 7 (47.2)
eosin yellow	362 ± 42 (64.5)	274 ± 13 (48.8)
hydrophobic		
ethyl eosin	354 ± 46 (63.1)	266 ± 20 (47.5)
protoporphyrin	468 ± 10 (83.4)	267 ± 6 (47.6)

[a] Cells were incubated in light at 1.5 mmol of photons m^{-2} s^{-1} at 30 °C for 30 min in the presence of the indicated photosensitizer at a final concentration of 10 μM. Cells were also incubated under these conditions in the presence of 200 μg mL^{-1} chloramphenicol (Cm). The activity of PSII prior to exposure to light was taken as 100%. Relative activities (in percent) are given in parentheses. Values are means ± SD of results from four independent experiments.

Photodamage to PSII Is Unaffected by the $^1O_2$-Generating System. We examined how photodamage to PSII varied with light intensity in the presence of chloramphenicol (Figure

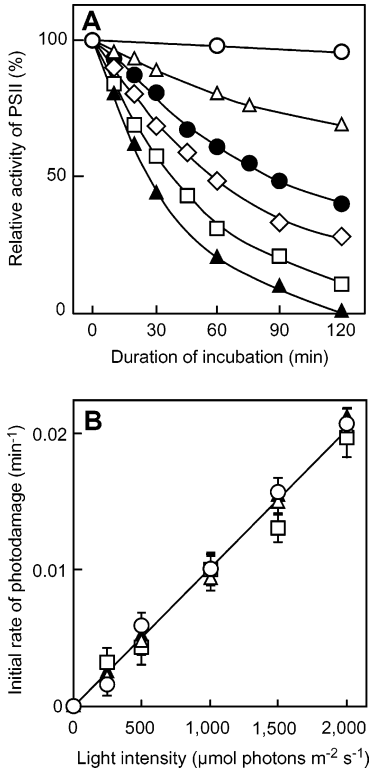

FIGURE 2: Relationship between light intensity and photodamage to PSII in *Synechocystis*. Cells were incubated in light at various intensities at 30 °C in the presence of chloramphenicol, and the initial rates of photodamage were determined as described in the text. (A) Profiles of photodamage to PSII in light at 0 (open circles), 250 (open triangles), 500 (closed circles), 1000 (open diamonds), 1500 (open squares), and 2000 (closed triangles) μmol of photons m^{-2} s^{-1}. (B) Relationship between light intensity and photodamage in the absence of reagents (open circles), in the presence of 10 μM rose bengal (open squares), in the presence of 10 μM DCMU (open triangles), and under anaerobic conditions in the presence of glucose, glucose oxidase, catalase, and DCMU (closed triangles; see text in details).

2A). The initial rate of photodamage to PSII in cells was proportional to the light intensity (Figure 2B, open circles). We next examined the initial rate of photodamage to PSII under different conditions. The relationship between the initial rate of photodamage and light intensity was unaffected when excess $^1O_2$ was produced as a result of the presence of rose bengal (Figure 2B, open squares). The relationship was also unaffected by the presence of DCMU, an herbicide that blocks the transport of electrons to the plastoquinone pool (Figure 2B, open triangles). The relationship was also unchanged when photodamage was induced under anaerobic conditions, which we achieved by using glucose oxidase and catalase to remove intracellular oxygen and DCMU to prevent the evolution of oxygen from PSII (Figure 2B, closed triangles). Thus, it appeared that the photodamage to PSII in vivo might be dependent solely on the intensity of light and might be independent of the presence of oxygen.

Inhibition of the Repair of Photodamaged PSII by the $^1O_2$-Generating System. We monitored inhibition of the repair by $^1O_2$ by examining the recovery of PSII activity after photodamage (Figure 3). Incubation of cells in light at 3.0 mmol of photons m^{-2} s^{-1} decreased the activity of PSII to approximately 20% of the original value. During subsequent exposure of cells to weak light at 70 μmol of photons of m^{-2} s^{-1}, the activity of PSII returned to the original level.

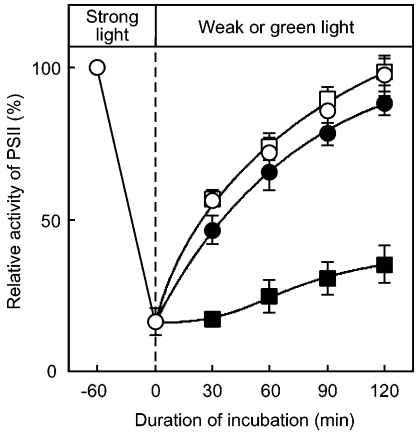

FIGURE 3: Effects of $^1O_2$ on the repair of photodamaged PSII in *Synechocystis*. Cells were incubated in light at 3 mmol of photons m^{-2} s^{-1} for 60 min without aeration to induce approximately 80% inactivation of PSII. Cells were then incubated with standard aeration in weak light at 70 μmol of photons m^{-2} s^{-1} in the presence of 10 μM rose bengal (open squares) or in its absence (open circles). Cells were also incubated in green light at 250 μmol of photons m^{-2} s^{-1} in the presence of 10 μM rose bengal (closed squares) or in its absence (closed circles). Values are means ± SD (bars) of results from four independent experiments.

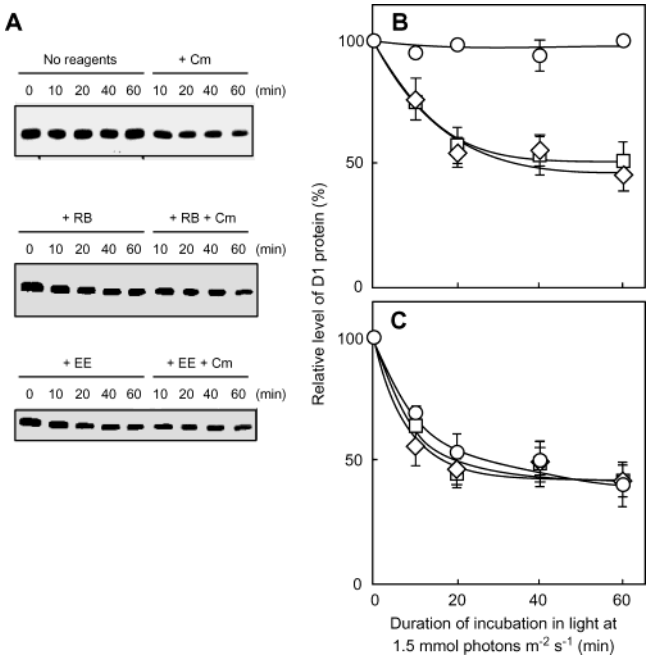

FIGURE 4: Effects of $^1O_2$ on the level of the D1 protein in *Synechocystis*. Cells were incubated in light at 1.5 mmol of photons m^{-2} s^{-1} at 30 °C with standard aeration in the absence (no reagents, open circles) and in the presence of 10 μM rose bengal (RB, open squares) and 10 μM ethyl eosin (EE, open diamonds). Cells were also exposed to light in the presence of each photosensitizer plus 200 μg mL^{-1} chloramphenicol (Cm). Thylakoid membranes were isolated from cells at the indicated times and subjected to Western blotting analysis. (A) Results of Western blotting. (B) Quantitation of the results shown in (A) in the absence of chloramphenicol. (C) Quantitation of the results shown in (A) in the presence of chloramphenicol. Values are means ± SD (bars) of results from three independent experiments.

At this light intensity, the presence of rose bengal barely affected the recovery of PSII activity. By contrast, in the presence of rose bengal, exposure of cells to green light at 250 μmol of photons m^{-2} s^{-1}, which excites rose bengal more efficiently than it excites Chls and phycobilisomes, resulted

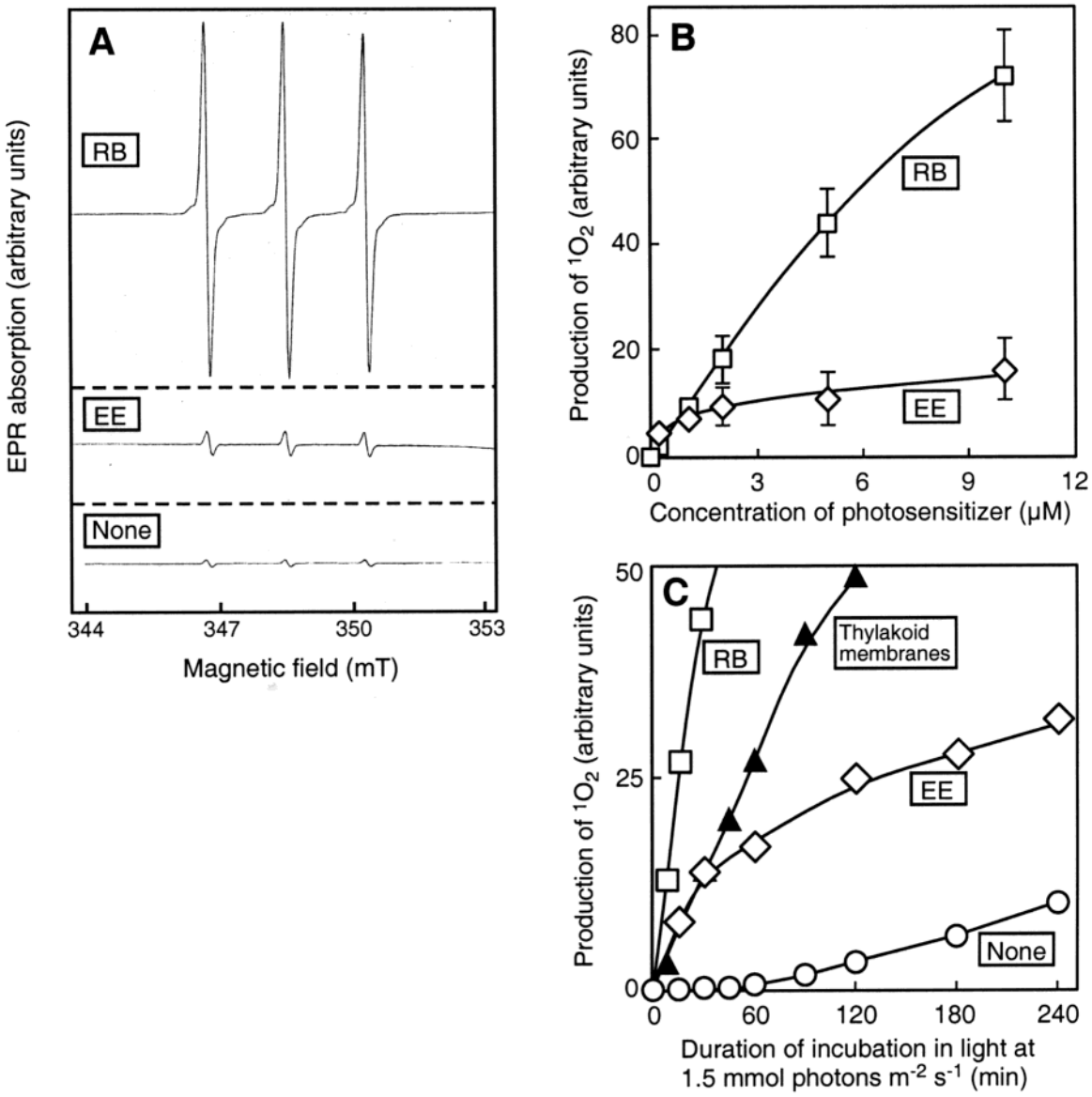

FIGURE 5: Intracellular production of $^1O_2$ in *Synechocystis* as detected by EPR. (A) EPR spectra of the nitroxide radical TEMPO in cells that had been incubated in light at 1.5 mmol of photons m^{-2} s^{-1} with standard aeration at 30 °C for 120 min in the presence of 10 μM rose bengal (RB) and 10 μM ethyl eosin (EE), respectively. (B) Changes in the level of the production of $^1O_2$ in the presence of various concentrations of rose bengal (open squares) and ethyl eosin (open diamonds) as determined from the EPR signals from TEMPO. (C) Changes in the level of the production of $^1O_2$ for various periods of time in the presence of 10 μM rose bengal (open squares) and 10 μM ethyl eosin (open diamonds) and in the absence of reagents (none, open circles). The production of $^1O_2$ in isolated thylakoid membranes was also shown (closed triangles). Values are means ± SD (bars) of results from three independent experiments.

in severe inhibition of the recovery of PSII activity. Exposure of cells to green light in the absence of rose bengal allowed PSII activity to recover to the original level.

Effects of the $^1O_2$-Generating System on the Level of the D1 Protein. We performed Western blotting to examine the changes in the level of the D1 protein during incubation in light at 1.5 mmol of photons m^{-2} s^{-1} in the presence of rose bengal and ethyl eosin (Figure 4). The presence of either rose bengal or ethyl eosin decreased the apparent level of the D1 protein (Figure 4B). The level of preexisting D1 protein decreased in the presence of chloramphenicol during incubation in light (Figure 4C). Neither the presence of rose bengal nor that of ethyl eosin enhanced the decrease in the level of the preexisting D1 protein (Figure 4C). These observations suggested that the production of $^1O_2$ in the cytosol and in thylakoid membranes inhibited the synthesis of the D1 protein but did not accelerate the degradation of this protein.

Production of $^1O_2$ by Photosensitizers. We examined the intracellular production of $^1O_2$ by monitoring EPR. Incubation of cells in light at 1.5 mmol of photons m^{-2} s^{-1} in the presence of rose bengal or of ethyl eosin plus TEMP resulted in an EPR spectrum that was characteristic of the stable nitroxide radical TEMPO, which is produced by the reaction of TEMP with $^1O_2$ (Figure 5A). We confirmed that Na_2Mn-EDTA quenched the EPR signals from TEMPO that was located outside cells (data not shown). In the presence of rose bengal, a hydrophilic photosensitizer, the level of $^1O_2$ increased in proportion to the concentration of photosensitizer added. In the presence of ethyl eosin, a hydrophobic photosensitizer, the level of $^1O_2$ increased more rapidly and reached a plateau value when the exogenous concentration

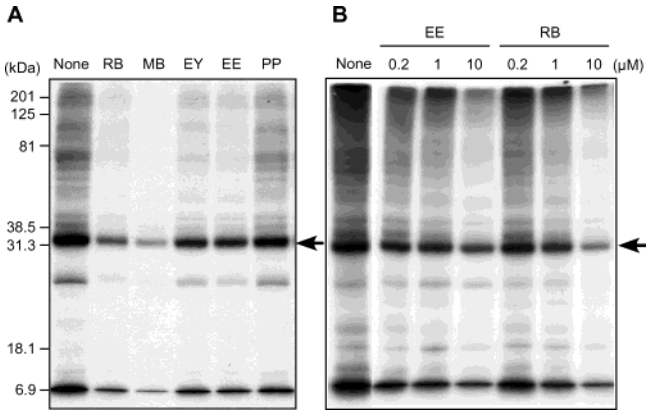

FIGURE 6: Effects of $^1O_2$ on the synthesis of the D1 protein de novo in *Synechocystis* as monitored in terms of the incorporation of radioactive [^{35}S]methionine into proteins in thylakoid membranes. (A) Labeled proteins in thylakoid membranes. Labeling was performed at 30 °C for 20 min in light at 1.5 mmol of photons m^{-2} s^{-1} in the absence of added reagents (none) and in the presence of 10 μM rose bengal (RB), 10 μM methylene blue (MV), 10 μM eosin yellow (EY), 10 μM ethyl eosin (EE), and 10 μM protoporphyrin (PP). The arrow indicates the D1 protein. (B) Labeled proteins in thylakoid membranes in the presence of different concentrations of rose bengal and ethyl eosin. The results shown are representative of the results from three independent experiments.

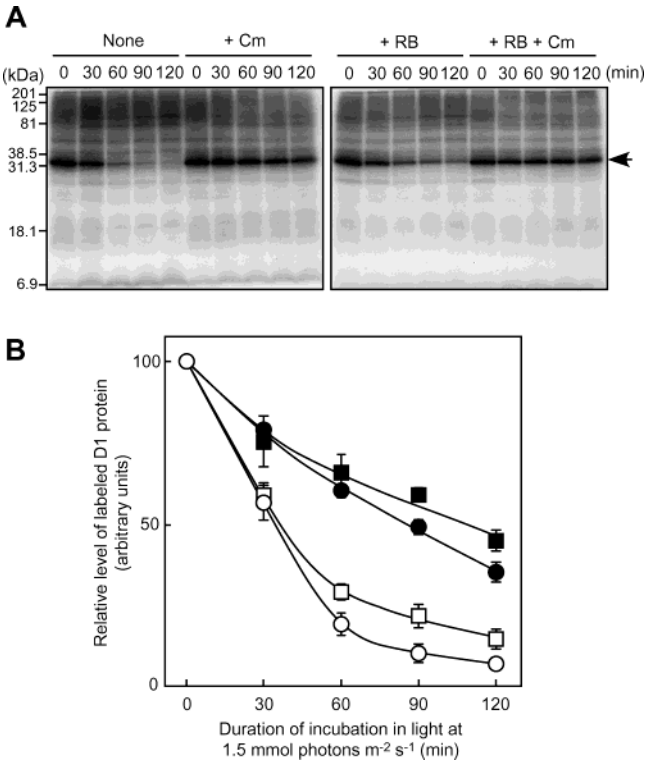

FIGURE 7: Effects of $^1O_2$ on the degradation of the D1 protein in *Synechocystis* as monitored in terms of the pulse−chase of proteins with [^{35}S]methionine in thylakoid membranes. Cells were pulse-labeled at 30 °C for 15 min in light at 1.5 mmol of photons m^{-2} s^{-1} and then supplemented with nonradioactive methionine. Pulse−chase was performed by incubation of labeled cells at 30 °C for designated times in light at 1.5 mmol of photons m^{-2} s^{-1} in the absence of added reagents (none) and in the presence of 5 μM rose bengal (RB). The pulse−chase experiments were also performed in the presence of 200 μg mL^{-1} chloramphenicol (Cm). (A) Results of pulse−chase. (B) Quantitation of the results shown in (A) and of two replicate experiments. The arrow indicates the D1 protein. Symbols: open circles, in the absence of added reagents; open squares, in the presence of rose bengal; closed circles, in the presence of chloramphenicol; closed squares, in the presence of rose bengal plus chloramphenicol.

of photosensitizer was 2 μM (Figure 5B). In the absence of photosensitizers, the level of $^1O_2$ increased to a much lesser degree during illumination, and in 4 h, it reached a level similar to that observed in the presence of 10 μM ethyl eosin in 20 min (Figure 5C). It should be noted that significant amounts of $^1O_2$ were produced in isolated thylakoid membranes even in the absence of photosensitizers (Figure 5C).

Inhibition of the Synthesis of the D1 Protein de Novo by the $^1O_2$-Generating System. We examined the effects of the $^1O_2$-generating system on the synthesis of the D1 protein de novo by monitoring the incorporation of [^{35}S]methionine into proteins in thylakoid membranes and, in particular, into the D1 protein (Figure 6). The synthesis of the D1 protein during exposure of cells to light at 1.5 mmol of photons m^{-2} s^{-1} was markedly suppressed in the presence of each of the photosensitizers examined. Furthermore, not only the synthesis of the D1 protein de novo but also the synthesis of almost all proteins in thylakoid membranes was suppressed by the presence of these photosensitizers (Figure 6A). The suppression of the synthesis of the D1 protein depended on the concentration of rose bengal (Figure 6B). In the presence of ethyl eosin, suppression was apparent at low exogenous concentrations, such as 0.2 μM, and it was not enhanced significantly above 1 μM (Figure 6B). This difference was reflected by the difference between the rates of the production of $^1O_2$ by the two types of photosensitizer (Figure 5B).

The chase of pulse-labeled proteins demonstrated that the presence of rose bengal did not accelerate the degradation of the D1 protein but slightly retarded its degradation during exposure of cells to light at 1.5 mmol of photons m^{-2} s^{-1} (Figure 7). The complete inhibition of the synthesis of proteins de novo with chloramphenicol retarded the degradation of the D1 protein to a larger extent. Ethyl eosin essentially had the same effects on the degradation of the D1 protein as did rose bengal (data not shown).

Effects of the $^1O_2$-Generating System on the Expression of psbA Genes. To determine whether $^1O_2$ might inhibit the synthesis of the D1 protein at the transcriptional level, we examined the effects of $^1O_2$ on the expression of the *psbA* genes, which encode the D1 protein. In *Synechocystis*, the D1 protein is encoded by a small multigene family that consists of the *psbA1*, *psbA2*, and *psbA3* genes (*29*). The *psbA1* gene is nonfunctional, while the *psbA2* and *psbA3* genes are expressed in response to light (*30*), and both encode the identical D1 protein.

We monitored changes in the combined levels of *psbA2* and *psbA3* mRNAs by Northern blotting (Figure 8). The level of *psbA* mRNAs increased during exposure of cells to light at 1.5 mmol of photons m^{-2} s^{-1}. The presence of rose bengal had no effect on the light-induced expression of *psbA* genes.

Effects of the $^1O_2$-Generating System on the Processing of the Precursor to the D1 Protein. Prior to maturation of the D1 protein in *Synechocystis*, the precursor to the D1 protein (pre-D1) is cleaved successively at two specific sites in its carboxy-terminal region (*20*). The levels of two forms of pre-D1, designated pre-D1-1 and pre-D1-2, increased during the exposure of cells to light at 1.5 mmol of photons m^{-2} s^{-1} (Figure 9). The presence of rose bengal decreased the levels of both precursors, a result that suggested that

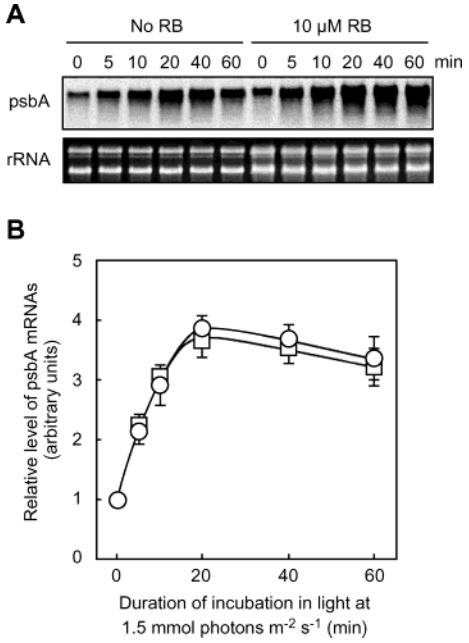

FIGURE 8: Effects of $^1O_2$ on the light-induced expression of *psbA* genes in *Synechocystis*. (A) Results of Northern blotting. (B) Quantitation of the results shown in (A) and of two replicate experiments. Prior to exposure to light, cells were incubated at 30 °C in darkness for 60 min. Cells were exposed to light at 1.5 mmol of photons m^{-2} s^{-1} with standard aeration in the presence of 10 μM rose bengal (RB; open squares) and in its absence (open circles). Total RNA (5 μg) was loaded in each lane of the gel. Levels of *psbA* mRNAs were normalized by reference to those of rRNA. Values in (B) are means ± SD (bars) of results from three independent experiments.

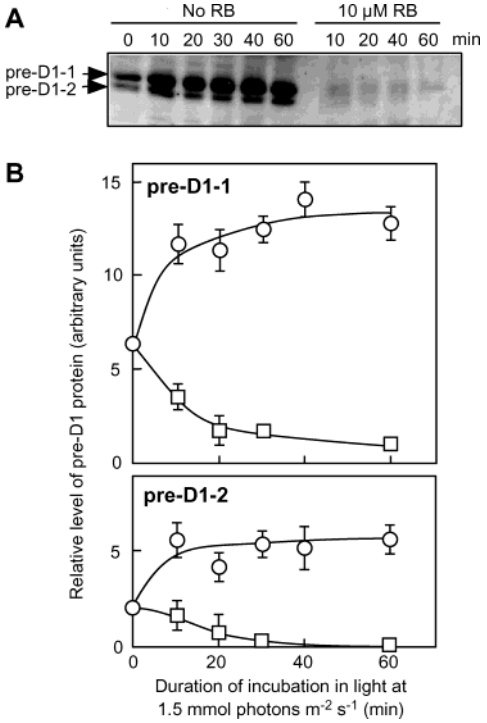

FIGURE 9: Effects of $^1O_2$ on the accumulation of pre-D1 proteins in *Synechocystis*. (A) Results of Western blotting. Cells were incubated in light at 1.5 mmol of photons m^{-2} s^{-1} at 30 °C with standard aeration in the presence of 10 μM rose bengal (RB; open squares) and in its absence (open circles). Thylakoid membranes were isolated from cells at the indicated times and subjected to Western blotting analysis. (B) Quantitation of the results shown in (A) and of two replicate experiments. Changes in the levels of pre-D1-1 and pre-D1-2 in the presence of 10 μM rose bengal (open squares) and in its absence (open circles) are shown. Values are means ± SD (bars) of results from three independent experiments.

inhibition by $^1O_2$ had already occurred before the processing steps. Considering these results together with the results of Northern blotting analysis, we postulated that the target of inhibition by $^1O_2$ during the synthesis of the D1 protein might be translation.

Effects of the $^1O_2$-Generating System on the Translation of psbA mRNAs. To identify the step in the translation of *psbA* mRNAs that was inhibited by $^1O_2$, we examined the effect of $^1O_2$ on the distribution of *psbA* mRNAs in membrane-bound and cytosolic polysomes, as well as in a polysome-free fraction (Figure 10). In *Synechocystis*, *psbA* mRNAs are first bound to ribosomes in the cytosol and then, after the nascent polypeptide reaches a certain length, *psbA* mRNA−ribosome complexes are targeted to thylakoid membranes where further elongation occurs (*31*).

After incubation of cells in darkness for 3 h, very low levels of *psbA* mRNAs were found together with cytosolic polysomes and membrane-bound polysomes and also in the polysome-free fraction (Figure 10, zero time). During the subsequent incubation of cells in light at 1.5 mmol of photons m^{-2} s^{-1}, the levels of *psbA* mRNAs associated with cytosolic polysomes and membrane-bound polysomes increased. The level of *psbA* mRNAs in the polysome-free fraction remained very low after illumination. The presence of rose bengal markedly decreased the association of *psbA* mRNAs with membrane-bound polysomes and increased the amount of *psbA* mRNAs that were associated with cytosolic polysomes. No *psbA* mRNAs accumulated in the polysome-free fraction in the presence of rose bengal. These observations suggest that the main target of inhibition by $^1O_2$ might be the elongation step of translation.

DISCUSSION

Effects of $^1O_2$ on the Photodamage to PSII. In the present study, we investigated the effects of $^1O_2$ on the photodamage to PSII that occurs in *Synechocystis* in strong light. To produce $^1O_2$ in the cells, we used hydrophilic and hydrophobic photosensitizers that produce $^1O_2$ in the cytosol and in thylakoid membranes, respectively. The production of $^1O_2$ either in the cytosol or in thylakoid membranes stimulated the apparent photodamage to PSII but did not enhance the photodamage when the repair of PSII was blocked by chloramphenicol (Figure 1 and Table 1). The repair of PSII was inhibited by the intracellular production of $^1O_2$ (Figure 3). These results suggest that $^1O_2$ might act by inhibiting the repair of photodamaged PSII in vivo.

Our finding that the initial rate of photodamage to PSII is proportional to light intensity suggests that photodamage to PSII is a purely light-dependent event (Figure 2). The proportionality between light intensity and photodamage to PSII has also been observed in plant leaves (*32*−*34*). This proportionality was totally unaffected when the transport of electrons was blocked by DCMU (Figure 2), suggesting that photodamage to PSII might be independent of the transport of electrons. Furthermore, the initial rate of photodamage to PSII was totally unaffected by production of excess $^1O_2$ or removal of intracellular O_2 (Figure 2). The production of $^1O_2$ in the cytosol by hydrophilic photosensitizers and also the production of $^1O_2$ in thylakoid membranes by hydro-

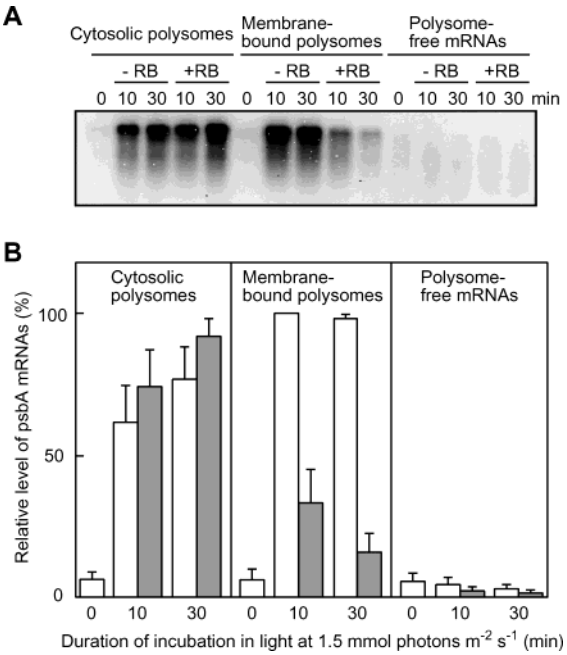

FIGURE 10: Effects of $^1O_2$ on the distribution of *psbA* mRNAs that were free or were associated with polysomes in *Synechocystis*. Cells were incubated at 30 °C in darkness for 3 h and then exposed to light at 1.5 mmol of photons m^{-2} s^{-1} for the indicated times with standard aeration in the presence of 10 μM rose bengal (RB) and in its absence. Cells were disrupted and membrane-bound polysomes, cytosolic polysomes, and polysome-free fractions were prepared. Northern blotting of *psbA* mRNAs in polysomes and polysome-free fractions was performed using a DNA fragment of the *psbA2* gene as probe. The RNA in each lane was derived from cells equivalent to 5 μg of Chl. (A) Gel electrophoretic pattern. (B) Quantitation of the results shown in (A) and of two replicate experiments. Key: open bars, levels of *psbA* mRNAs in the absence of rose bengal; shaded bars, levels of *psbA* mRNAs in the presence of rose bengal. Values are means ± SD (bars) of results from three independent experiments.

phobic photosensitizers failed to affect the photodamage to PSII (Table 1). These findings indicate that the production of $^1O_2$ in close proximity to the reaction center of PSII might not cause the damage to PSII. All of these observations suggest that $^1O_2$ might not be responsible for photodamage to PSII.

As proposed by Anderson in 2001, oxygen molecules might be sheltered from the reaction center Chl (P680) by a hydrophobic environment around the reaction center, which would prevent the formation of $^1O_2$, and oxygen molecules that are evolved at the manganese cluster might be directed outward by a specific oxygen channel (*35*, *36*). $^1O_2$ might be generated from nonfunctional PSII, in which such an oxygen channel has already been disrupted as a result of photodamage (*36*), as observed previously (*10*−*12*). In fact, we found that a significant amount of $^1O_2$ was produced in isolated thylakoid membranes during illumination, whereas much less amount of $^1O_2$ was produced by intact cells (Figure 5). The production of $^1O_2$ in cells might have been derived, in part, from the photodamaged PSII. However, we cannot exclude the possibility that the high rate of the production of $^1O_2$ in thylakoid membranes might be due to the free access of the spin trap to the membranes or to the production of $^1O_2$ by free Chls produced during the isolation.

Inhibition by $^1O_2$ of the Repair of Photodamaged PSII. The apparent photodamage to PSII in vivo reflects the balance between actual photodamage to PSII and the repair of photodamaged PSII. The present study demonstrated that $^1O_2$ stimulates the apparent photodamage by inhibiting the repair of photodamaged PSII in vivo (Figures 1 and 3). Recently, we demonstrated that other ROS, such as H_2O_2 and O_2^-, act primarily by inhibiting the repair of photodamaged PSII (*21*). Thus, it seems likely that oxidative stress due to ROS might, in general, be primarily responsible for the inhibition of repair. Such a repair-inhibiting effect has also been demonstrated in the case of the effects of salt stress on PSII (*37*, *38*). Salt stress due to NaCl inhibits the repair of photodamaged PSII but does not accelerate damage to PSII directly.

Suppression by $^1O_2$ of the Synthesis of Proteins. Western blotting revealed that the production of $^1O_2$ in the cytosol and in thylakoid membranes did not accelerate the degradation of preexisting D1 protein when repair was blocked (Figure 4). In addition, the pulse−chase experiments demonstrated that the degradation of the newly synthesized D1 protein was not accelerated by the production of $^1O_2$ (Figure 7). The retardation in the degradation of the D1 protein in the presence of photosensitizers or chloramphenicol might be explained by the perturbation of the coordination between the synthesis of the D1 protein de novo and its degradation, as observed previously (*39*). These observations are consistent with our finding that the production of $^1O_2$ did not accelerate the photodamage to PSII.

Labeling of proteins in vivo demonstrated clearly that $^1O_2$ acts by suppressing the synthesis of the D1 protein de novo (Figure 6). The production of $^1O_2$ both in the cytosol and in thylakoid membranes suppressed the synthesis of the D1 protein de novo. It is noteworthy that not only the synthesis of the D1 protein de novo but also the synthesis of almost all other proteins in thylakoid membranes was suppressed by the presence of $^1O_2$ (Figure 6). This observation suggests that the target of suppression by $^1O_2$ might be a process common to the synthesis of all proteins. Northern blotting indicated that the presence of $^1O_2$ had no effect on the light-induced expression of *psbA* genes (Figure 8). Western blotting of pre-D1 revealed that the processing of pre-D1, which is a specific posttranslational event that is necessary for generation of the D1 protein, was not the target of $^1O_2$ (Figure 9). Thus, it seems likely that the primary target of $^1O_2$ might be translation.

Translation Elongation as the Primary Target of $^1O_2$. Analysis of polysomes indicated that $^1O_2$ markedly decreased the level of *psbA* mRNA−ribosome complexes that were associated with thylakoid membranes (Figure 10). In *Synechocystis*, *psbA* mRNAs associate with ribosomes in the cytosol (at the initiation of translation), and then elongation starts. When the molecular mass of the polypeptide reaches 17 kDa, the *psbA* mRNA−ribosome complexes are targeted to thylakoid membranes where further elongation occurs (*31*). Thus, a decreased level of membrane-bound complexes suggests that the primary site of suppression by $^1O_2$ might be the elongation step of translation. To our knowledge, this is the first report that demonstrates that $^1O_2$ inhibits the translational machinery.

We demonstrated recently that another ROS, H_2O_2, inhibits the translation of mRNA into proteins by suppressing elongation strongly and initiation to a lesser extent (*21*). It appears that $^1O_2$ and H_2O_2 might inactivate the translational

machinery in a similar manner.

The generation of the various ROS is promoted under excess light, but their major sources are different. $^1O_2$ is generated by the transfer of energy from excited pigments, such as Chls, in the light-harvesting complexes (*2*, *40*) and from excited Fe-S centers in photosystem I (*41*). $^1O_2$ might also be generated from nonfunctional PSII that has been photodamaged (*36*). The $^1O_2$ formed is a metastable entity that can diffuse through aqueous and nonpolar environments (*2*). H_2O_2, a stable entity, is generated mainly by the enzymatic or spontaneous reduction of O_2^-, which is generated most abundantly on the acceptor side of photosystem I (*3*). $^1O_2$ oxidizes histidine preferentially and methionine and tryptophan to a lesser extent (*2*). H_2O_2 is converted to the most toxic ROS, ·OH, via reactions with reduced metal ions (*3*) and non-heme iron (*16*). It is conceivable that the ·OH formed in this way might oxidize or modify the side chains of amino acid residues and, preferentially, those of histidine, arginine, and proline (*16*). It is also possible that $^1O_2$ and H_2O_2 might have the same effects, exerted via the modification of the same amino acid residues. In any case, the major target of such ROS might well be specific amino acid residues in proteins that are involved in elongation in the ribosome complex.

During translation in *Escherichia coli*, H_2O_2 inactivates elongation via carbonylation of elongation factor G (*42*). Elongation factor G has also been identified as one of the proteins that are most susceptible to oxidation in a mutant of *E. coli* that lacks a superoxide dismutase (*43*). Treatment of rat liver with the oxidant cumene hydroperoxide results in inhibition of the synthesis of proteins via inactivation of elongation factor 2 (*44*). Thus, elongation factors are the most probable candidates for targets of $^1O_2$ and H_2O_2 in *Synechocystis*.

In conclusion, it appears that both $^1O_2$ and H_2O_2 also act by inactivating the translational machinery, thereby repressing the synthesis de novo of proteins and, in particular, of the D1 protein, which is required for the repair of photodamaged PSII. The resulting inhibition of repair might be in part responsible for decrease in the activity of PSII.

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REFERENCES

1. Halliwell, B., and Gutteridge, J. M. C. (1990) Role of free radicals and catalytic metal ions in human disease: an overview, *Methods Enzymol. 186*, 1−88.
2. Knox, J. P., and Dodge, A. D. (1985) Singlet oxygen and plants, *Phytochemistry 24*, 889−896.
3. Asada, K. (1996) Radical Production and Scavenging in the Chloroplasts, in *Photosynthesis and the Environment* (Baker, N. R., Ed.) pp 123−150, Kluwer Academic Publishers, Dordrecht, The Netherlands.
4. Asada, K. (1999) The water−water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons, *Annu. Rev. Plant Physiol. Plant Mol. Biol. 50*, 601−639.
5. Prásil, O., Adir, N., and Ohad, I. (1992) Dynamics of Photosystem II: Mechanism of Photoinhibition and Recovery Processes, in *Topics in Photosynthesis, The Photosystems: Structure, Function and Molecular Biology* (Barber, J., Ed.) Vol. 11, pp 295−348, Elsevier Science Publishers, Amsterdam, The Netherlands.
6. Aro, E.-M., Virgin, I., and Andersson, B. (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover, *Biochim. Biophys. Acta 1143*, 113−134.
7. Andersson, B., and Aro, E.-M. (2001) Photodamage and D1 Protein Turnover in Photosystem II, in *Regulation of Photosynthesis* (Aro, E.-M., and Andersson, B., Eds.) pp 377−393, Kluwer Academic Publishers, Dordrecht, The Netherlands.
8. Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.-M., and Andersson, B. (1992) The reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced Q_A species promote chlorophyll triplet formation, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1408−1412.
9. Keren, N., Berg, A., van Kan, P. J. M., Levanon, H., and Ohad, I. (1997) Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: The role of back electron flow, *Proc. Natl. Acad. Sci. U.S.A. 94*, 1579−1584.
10. Telfer, A., Bishop, S. M., Phillips, D., and Barber, J. (1994) The isolated photosynthetic reaction center of PS II as a sensitiser for the formation of singlet oxygen; detection and quantum yield determination using a chemical trapping technique, *J. Biol. Chem. 269*, 13244−13253.
11. Hideg, E., Spetea, C., and Vass, I. (1994) Singlet oxygen and free radical production during acceptor- and donor-side-induced photoinhibition. Studies with spin trapping EPR spectroscopy, *Biochim. Biophys. Acta 1186*, 143−152.
12. Hideg, E., Kálai, T., Hideg, K., and Vass, I. (1998) Photoinhibition of photosynthesis *in vivo* results in singlet oxygen production: detection via nitroxide-induced fluorescence quenching in broad bean leaves, *Biochemistry 37*, 11405−11411.
13. Okada, K., Ikeuchi, M., Yamamoto, N., Ono, T., and Miyao, M. (1996) Selective and specific cleavage of the D1 and D2 proteins of photosystem II by exposure to singlet oxygen: factors responsible for the susceptibility to cleavage of the proteins, *Biochim. Biophys. Acta 1274*, 73−79.
14. Ananyev, G., Wydrzynski, T., Renger, G., and Klimov, V. (1992) Transient peroxide formation by the manganese-containing, redox-active donor side of photosystem II upon inhibition of O_2 evolution with lauroylcholine chloride, *Biochim. Biophys. Acta 1100*, 303−311.
15. Chen, G.-X., Kazimir, J., and Cheniae, G. M. (1992) Photoinhibition of hydroxylamine-extracted photosystem II membranes: studies of the mechanism, *Biochemistry 31*, 11072−11083.
16. Miyao, M., Ikeuchi, M., Yamamoto, N., and Ono, T. (1995) Specific degradation of the D1 protein of photosystem II by treatment with hydrogen peroxide in darkness: implication for the mechanism of degradation of the D1 protein under illumination, *Biochemistry 34*, 10019−10026.
17. Ohad, I., Kyle, D. J., and Arntzen, C. J. (1984) Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptide in chloroplast membranes, *J. Cell Biol. 99*, 481−485.
18. Mattoo, A. K., Marder, J. B., and Edelman, M. (1989) Dynamics of the photosystem II reaction center, *Cell 56*, 241−246.
19. Anbudurai, P. R., Mor, T. S., Ohad, I., Shestakov, S. V., and Pakrasi, H. B. (1994) The *ctpA* gene encodes the C-terminal processing protease for the D1 protein of the photosystem II reaction center complex, *Proc. Natl. Acad. Sci. U.S.A. 91*, 8082−8086.
20. Inagaki, N., Yamamoto, Y., and Satoh, K. (2001) A sequential two-step proteolytic process in the carboxy-terminal truncation of precursor D1 protein in *Synechocystis* sp. PCC 6803, *FEBS Lett. 509*, 197−201.
21. Nishiyama, Y., Yamamoto, H., Allakhverdiev, S. I., Inaba, M., Yokota, A., and Murata, N. (2001) Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery, *EMBO J. 20*, 5587−5594.
22. Gombos, Z., Wada, H., and Murata, N. (1994) The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane lipids: a mechanism of chilling tolerance, *Proc. Natl. Acad. Sci. U.S.A. 91*, 8787−8791.
23. Arnon, D. I., McSwain, B. D, Tsujimoto, H. Y., and Wada, K. (1974) Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll *a* and removal of phycocyanin, *Biochim. Biophys. Acta 357*, 231−245.
24. Lion, Y., Delmelle, M., and van de Vorst, A. (1976) New method of detecting singlet oxygen production, *Nature 263*, 442−443.

25. Lion, Y., Gandin, E., and van de Vorst, A. (1980) On the production of nitroxide radicals by singlet oxygen reaction: An EPR study, *Photochem. Photobiol. 31*, 305−309.
26. Blumwald, E., Mehlhorn, R. J., and Packer, L. (1983) Studies of osmoregulation in salt adaptation of cyanobacteria with ESP spin-probe techniques, *Proc. Natl. Acad. Sci. U.S.A. 80*, 2599−2602.
27. Nishiyama, Y., Los, D. A., and Murata, N. (1999) PsbU, a protein associated with photosystem II, is required for the acquisition of cellular thermotolerance in *Synechococcus* species PCC 7002, *Plant Physiol. 120*, 301−308.
28. Los, D. A., Ray, M., and Murata, N. (1997) Differences in the control of the temperature-dependent expression of four genes for desaturases in *Synechocystis* sp. PCC 6803, *Mol. Microbiol. 25*, 1167−1175.
29. Jansson, C., Debus, R. J., Osiewacz, H. D., Gurevitz, M., and McIntosh, L. (1987) Construction of an obligate photoheterotrophic mutant of the cyanobacterium *Synechocystis* 6803. Inactivation of the *psbA* gene family, *Plant Physiol. 85*, 1021−1025.
30. Tyystjärvi, T., Tyystjärvi, E., Ohad, I., and Aro, E.-M. (1998) Exposure of *Synechocystis* 6803 cells to series of single turnover flashes increases the *psbA* transcript level by activating transcription and down-regulating *psbA* mRNA degradation, *FEBS Lett. 436*, 483−487.
31. Tyystjärvi, T., Herranen, M., and Aro, E.-M. (2001) Regulation of translation elongation in cyanobacteria: membrane targeting of the ribosome nascent-chain complexes controls the synthesis of D1 protein, *Mol. Microbiol. 40*, 476−484.
32. Park, Y.-I., Chow, W. S., and Anderson, J. M. (1995) Light inactivation of functional photosystem II in leaves of peas grown in moderate light depends on photon exposure, *Planta 196*, 401−411.
33. Tyystjärvi, E., and Aro, E.-M. (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity, *Proc. Natl. Acad. Sci. U.S.A. 93*, 2213−2218.
34. Tyystjärvi, E., Kairavuo, M., Pätsikkä, E., Keränen, M., Khriachtchev, L., Tuominen, I., Guiamet, J. J., and Tyystjärvi, T. (2001) The Quantum Yield of Photoinhibition Is the Same in Flash Light and under Continuous Illumination: Implication for the Mechanism, in *Proceedings of the 12th International Congress of Photosynthesis* (Critchley, C., Ed.) S8-P032, CSIRO Publishing, Melbourne, Australia.
35. Wydrzynski, T., Hillier, W., and Messinger, J. (1996) On the functional significance of substrate accessibility in the photosynthetic water oxidation mechanism, *Physiol. Plant 96*, 342−350.
36. Anderson, J. M. (2001) Does functional photosystem II complex have an oxygen channel? *FEBS Lett. 488*, 1−4.
37. Allakhverdiev, S. I., Nishiyama, Y., Suzuki, I., Tasaka, Y., and Murata, N. (1999) Genetic engineering of the unsaturation of fatty acids in membrane lipids alters the tolerance of *Synechocystis* to salt stress, *Proc. Natl. Acad. Sci. U.S.A. 96*, 5862−5867.
38. Allakhverdiev, S. I., Nishiyama, Y., Miyairi, S., Yamamoto, H., Inagaki, N., Kanesaki, Y., and Murata, N. (2002) Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of *psbA* genes in *Synechocystis*, *Plant Physiol. 130*, 1443−1453.
39. Komenda, J., and Barber, J. (1995) Comparison of *psbO* and *psbH* deletion mutants of *Synechocystis* PCC 6803 indicates that degradation of D1 protein is regulated by the Q_B site and dependent on protein synthesis, *Biochemistry 34*, 9625−9631.
40. Zolla, L., and Rinalducci, S. (2002) Involvement of active oxygen species in degradation of light-harvesting proteins under light stresses, *Biochemistry 41*, 14391−14402.
41. Chung, S. K., and Jung, J. (1995) Inactivation of the acceptor side and degradation of the D1 protein of photosystem II by singlet oxygen photogenerated from the outside, *Photochem. Photobiol. 61*, 383−389.
42. Tamarit, J., Cabiscol, E., and Ros, J. (1998) Identification of the major oxidatively damaged proteins in *Escherichia coli* cells exposed to oxidative stress, *J. Biol. Chem. 273*, 3027−3032.
43. Dukan, S., and Nyström, T. (1999) Oxidative stress defense and deterioration of growth-arrested *Escherichia coli* cells, *J. Biol. Chem. 274*, 26027−26032.
44. Ayala, A., Parrado, J., Bougria, M., and Machado, A. (1996) Effect of oxidative stress, produced by cumene hydroperoxide, on the various steps of protein synthesis, *J. Biol. Chem. 271*, 23105−23110.

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