Characterization of damage to photosystems I and II in a cyanobacterium lacking detectable iron superoxide dismutase activity

(gene replacement/photoacoustics/photoinhibition/sodB/photosynthesis)

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ABSTRACT   The enzyme superoxide dismutase is ubiquitous in aerobic organisms where it plays a major role in alleviating oxygen-radical toxicity. An insertion mutation introduced into the iron superoxide dismutase locus (designated sodB) of the cyanobacterium Synechococcus sp. PCC 7942 created a mutant strain devoid of detectable iron superoxide dismutase activity. Both wild-type and mutant strains exhibited similar photosynthetic activity and viability when grown with 17 μmol·m^{-2}·s^{-1} illumination in liquid culture supplemented with 3% carbon dioxide. In contrast, the sodB mutant exhibited significantly greater damage to its photosynthetic system than the wild-type strain when grown under increased oxygen tension or with methyl viologen. Although damage occurs at both photosystems I and II, it is primarily localized at photosystem I in the sodB mutant. Growth in 100% molecular oxygen for 24 hr decreased photoacoustically measured energy storage in 3-(3,4-dichlorophenyl)-1,1-dimethylurea and abolished the fluorescence state 2 to state 1 transition in the sodB mutant, indicating interruption of cyclic electron flow around photosystem I. Analysis of the flash-induced absorption transient at 705 nm indicated that the interruption of cyclic electron flow occurred in the return part of the cycle, between the two [4 Fe—4 S] centers of photosystem I, F_A and F_B, and cytochrome f. Even though the sodB mutant was more sensitive to damage by active oxygen than wild-type cells, both strains were equally sensitive to the photoinhibition of photosystem II caused by exposure to strong light.

The photoproduction of a variety of active species of oxygen is inevitable in photosynthetic organisms. Activation of O_2 in the oxygen-evolving photosynthetic system can occur primarily through two pathways. The superoxide anion radical (O_2^-) can be produced through the interaction of O_2 with electron acceptors of photosystem I (PSI), and singlet dioxygen ($^1O_2$) can result through the interaction of O_2 with excited triplet-state chlorophyll in the chlorophyll antennae of PSI and photosystem II (PSII). Photoreduction of O_2 by PSI is a common feature of photosynthetic electron flow in chloroplasts and cyanobacteria, particularly when CO_2 is limiting to photosynthesis (1, 2).

O_2^- is not particularly reactive with cellular components but can interact with H_2O_2 in a Haber–Weiss type reaction to form the highly reactive hydroxyl radical (OH·). Once produced, the hydroxyl radical can react indiscriminately to cause lipid peroxidation, the denaturation of proteins, and mutational damage to DNA. Therefore, it is imperative to the survival of photosynthetic organisms to scavenge H_2O_2 and O_2^- immediately to prevent photooxidative damage (3).

Superoxide dismutases (SODs) are metalloenzymes of 17–85 kDa that are ubiquitous in aerobic organisms and function by catalyzing the dismutation of O_2^- to H_2O_2 and O_2. There are three types of SOD, which can be distinguished by the prosthetic metals present at the active site: iron (FeSOD), manganese (MnSOD), and copper/zinc (Cu/ZnSOD). The FeSOD enzyme has been detected in cyanobacteria and other prokaryotes as well as in the plastids of some plants (4, 5). MnSOD has been detected in some, but not all, cyanobacteria and is also widely distributed among prokaryotic and eukaryotic organisms. In cyanobacteria, the FeSOD is localized in the cytosol, whereas the MnSOD is found in the thylakoid membranes (5). Sequence homologies suggest that MnSOD and FeSOD evolved from a common ancestral form in early prokaryotes, whereas the Cu/ZnSOD appears to have evolved independently in the green plant line. The Cu/ZnSOD is usually present in the cytosol and some plants also contain a chloroplastic version (6).

All three SOD types are highly reactive with O_2^-, and their activity is substrate-limited under physiological conditions (3). In plants and cyanobacteria, SOD activity has been shown (4, 7) to change dramatically in response to conditions that favor formation of O_2^-. The role of toxic O_2 species in photooxidative damage and cell death has been well established in cyanobacteria (7).

In the present study, the cloned sodB gene from Synechococcus sp. PCC 7942 (8) has been used to interrupt the native FeSOD gene by the introduction of a spectinomycin-resistance gene cartridge into the coding region. The sensitivity of the resulting sodB mutant to oxygen and light has been characterized.

MATERIALS AND METHODS

Strains and Culture Conditions. Synechococcus sp. PCC 7942 was grown axenically in liquid and solid (1% Bactoagar) BG-11 medium (9) buffered to pH 8.0 with 20 mM Hepes. Liquid cultures were grown at 29°C in 60-ml glass tubes sparged with 3% CO_2 in air with incandescent light at 17 μmol·m^{-2}·s^{-1}.

Insertional Inactivation of the sodB Gene. A 2.1-kilobase (kb) Pst I fragment, encoding the sodB gene, had been cloned into the Pst I site of pUC18 (8). Plasmid DNA from this clone,

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designated pFSP3, was isolated from a *dam⁻ Escherichia coli* strain and used in subsequent constructions. A 2.0-kb *Bam*HI fragment containing the spectinomycin-resistance gene from pHP45Ω (10) was ligated into pFSP3 digested with *Bcl* I and transformed into *E. coli* DH5α. The resulting clone, designated pFSP274-3, was used to transform *Synechococcus* sp. PCC 7942 to spectinomycin resistance by the method of Laudenbach *et al.* (11).

SOD Enzyme Activity Assays. Cyanobacterial protein extracts were prepared by harvesting 100 ml of cells by centrifugation at 3500 × *g* for 5 min. The pellet was washed and resuspended in 3 ml of 50 mM potassium phosphate, pH 7.8/0.5 mM EDTA. This suspension was passed through a French pressure cell at 16,000 psi (1 psi = 6.9 kPa) and centrifuged at 6000 × *g* for 10 min to remove large debris and unbroken cells. The supernatant was recentrifuged at 100,000 × *g* for 60 min in a Ti50 fixed-angle rotor (Beckman). The supernatant, designated the soluble protein fraction, was used immediately in SOD assays. The pellet, designated the membrane fraction, was resuspended by sonication in a minimum volume of 50 mM potassium phosphate, pH 7.8/0.5 mM EDTA and immediately used in SOD enzyme assays.

SOD activity was assayed on the ability of SOD to inhibit the O_2^--mediated reduction of nitro blue tetrazolium (12). MnSOD, which has been reported to be partially or fully insensitive to H_2O_2 treatment in cyanobacteria (5), was determined by adding 1 mM H_2O_2 and 5 mM potassium cyanide to the reaction mixture. The potassium cyanide was added to inhibit the breakdown of H_2O_2 by catalases present in the crude protein extracts. One unit of SOD activity is defined as the amount that decreases the rate of nitro blue tetrazolium reduction by 50%. The protein content of the cell-free extracts was determined with the bicinchoninic acid protein-assay reagent kit (Pierce).

Measurements of Photosynthetic Electron Flow. Photoacoustic measurements of energy storage associated with photosynthetic electron flow were made in intact cells, as described (13). The photoacoustic measuring beam was modulated at 150 Hz. A Clark-type oxygen electrode (Rank Brothers, Cambridge, U.K.) was used to measure the rates of photosynthetic oxygen evolution by intact cells suspended in growth medium.

Measurements of Chlorophyll Fluorescence. Induction of chlorophyll fluorescence from PSII was observed in intact cells at room temperature in the presence of 25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Actinic light (442 nm) was from a HeCd laser (Liconix, Sunnyvale, CA) at 250 μmol·m⁻²·s⁻¹. The photomultiplier detector (Photonics R928, Hamamatsu, Middlesex, NJ) was protected by a red glass cut-off filter (RG665, Schott Glass Technologies, Durea, PA). Fluorescence transients were collected and analyzed by using a commercially available microcomputer program and a fast analog-to-digital converter card (Q_A Data, Turku, Finland) described elsewhere (14). State transitions were controlled as in Satoh and Fork (15).

Light-Induced Absorbance Changes. Light-induced absorbance transients were observed in intact cells at room temperature with a laboratory-built spectrophotometer combined with a digital signal averager (model 1010; Nicolet). Measurements of cytochromes *f* and *c553* at 420 nm were made as described (11). For measurements of P700 at 705 nm, the spectrophotometer was operated in split-beam mode, and the measuring beam was modulated by a mechanical chopper at 150 Hz. The signal from the reference photomultiplier was subtracted from the sample photomultiplier signal by a lock-in amplifier (SR510; Stanford Research Systems, Sunnyvale, CA) before being fed into the signal averager. The actinic lamp was fitted with blue and blue-green glass filters (Corning 5-59 and 4-98) for these measurements, and the sample photomultiplier was protected by a red glass cut-off

filter (RG 695). The sample was placed 23 cm above the detector to eliminate pick-up of background chlorophyll fluorescence.

RESULTS

Insertional Inactivation of the *sodB* Gene. In *Synechococcus* sp. PCC 7942 the *sodB* gene is present in a single copy in the chromosome and is transcribed as a monocistronic mRNA (8). A restriction map and the site of insertion used in the inactivation of *sodB* is shown in Fig. 1A.

Initial analysis of transformants by DNA gel-blot analysis indicated that these colonies were heterozygous for the *sodB* inactivation construct (results not shown). This condition results because cyanobacteria contain multiple copies of their chromosome in individual cells. Restreaking attempts to segregate the mutant allele of *sodB* to a homozygous condition were successful only after the BG-11 agar medium was supplemented with 10 mM sodium bicarbonate and catalase (10 units per ml; Sigma). DNA gel-blot hybridization (Fig. 1B) showed that the *sodB* probe hybridized to a 2.1-kbp *Pst* I (lane 1) and an 8.0-kbp *Eco*RI (lane 3) fragment of chromosomal DNA from wild-type cells. The same probe hybridized to a 4.1-kbp *Pst* I (lane 2) and a 10.0-kbp *Eco*RI (lane 4) fragment of DNA from the *sodB* mutant, as expected for an interrupted *sodB* gene (see Fig. 1A). Lack of a 2.1-kbp *Pst* I and 8.0-kbp *Eco*RI fragment in the *sodB* mutant DNA indicates that the wild-type allele of *sodB* is absent in this mutant strain.

SOD Enzyme Activity. Table 1 shows that the H_2O_2-sensitive FeSOD enzyme was the major SOD activity in the soluble protein fraction in wild-type cells of *Synechococcus* sp. PCC 7942. Conversely, most SOD activity in the membrane fraction was from the H_2O_2-insensitive MnSOD. The absence of H_2O_2-sensitive SOD activity in the soluble fraction of the *sodB* mutant confirmed the phenotype of the mutant. However, high levels of MnSOD activity were still detected in the membrane fraction of the *sodB* mutant.
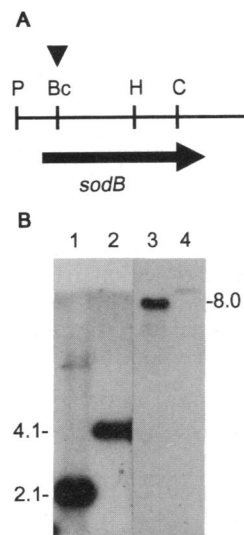

FIG. 1. (A) Restriction map of *sodB* gene. A 2.0-kb *Bam*HI fragment containing a spectinomycin resistance gene was cloned into the *Bcl* I site (designated by arrow), and the resulting clone was used to interrupt *sodB* via a homologous, double-recombination event in *Synechococcus* sp. PCC 7942. P, *Pst* I; Bc, *Bcl* I; H, *Hind*III; C, *Cla* I. (B) Southern blot of genomic DNA from wild-type (lanes 1 and 3) and mutant *sodB* (lanes 2 and 4) cells of *Synechococcus* sp. PCC 7942. DNA was digested with *Pst* I (lanes 1 and 2) or *Eco*RI (lanes 3 and 4), electrophoresed in a 0.7% agarose gel, transferred to nitrocellulose, and probed with a ³²P-labeled *sodB* probe. Size markers are in kilobase pairs (kbp).

Table 1. Characterization of cyanobacterial SOD activity

| Organism | Cellular fraction | SOD activity, units per mg of protein | |
		H_2O_2 insensitive	H_2O_2 sensitive
Synechococcus	Soluble	0.27	6.25
PCC 7942	Membrane	6.35	0.80
Synechococcus	Soluble	0.60	0.05
PCC 7942 *sodB*	Membrane	9.50	0.75
P. boryanum	Soluble	3.40	5.00
UTEX 485	Membrane	10.10	0.20

Levels of SOD activity in *Plectonema boryanum* UTEX 485 were included as controls because both FeSOD and MnSOD proteins have been localized and characterized in this cyanobacterium (5, 16).

Effects of Elevated O_2 on Fluorescence Characteristics of PSII. Liquid cultures of the wild-type and *sodB* mutant grown at 29°C were sparged with pure O_2 for 24 hr under weak incandescent illumination (17 μmol·m^{-2}·s^{-1}). Before and after this treatment, chlorophyll *a* induction transients were measured in both fluorescence state 1 and fluorescence state 2 (Table 2). The treatment decreased photochemical activity of PSII in states 1 and 2 in both strains but decreased this activity more so in the *sodB* mutant. More dramatic was the effect of O_2 treatment on the transition from fluorescence state 2 to fluorescence state 1, quantified as (F_m in state 1 − F_m in state 2/F_m in state 2). In wild-type cells, 24 hr of treatment decreased the state transition by 28%. In the *sodB* mutant, the same duration of treatment abolished the state transition completely.

Effects of Elevated O_2 on Electron Flow Through PSI. Electron flow through PSI was assessed by photoacoustic measurements of energy storage in 700-nm light with the addition of 25 μM DCMU. Most energy storage seen under these conditions results from cyclic electron flow around PSI (13). In the wild-type strain, photoacoustic measurements of PSI energy storage increased slightly from 12.9% to 13.7% after 24 hr of treatment in 100% O_2 and weak light. Conversely, the same treatment decreased PSI energy storage from 12.0% to 6.3% in the *sodB* mutant. These values are all means of eight samples with a SD of ±3.4% or less.

Electron flow through PSI was also measured spectroscopically. Flash-driven oxidation and subsequent dark reduction of the reaction center of PSI (P700) were measured in whole cells using flash-induced changes in absorbance at 705 nm (Fig. 2). In these measurements, the slope of the light-induced decrease in absorbance at 705 nm was interpreted to indicate the rate of P700 oxidation, the extent of the light-induced decrease in absorbance was interpreted to represent the quantity of photooxidizable P700, and the slope of the initial,

Table 2. Effects of elevated O_2 and 17 μmol·m^{-2}·s^{-1} white light on induction of room-temperature chlorophyll fluorescence (685 nm) in 25 μM DCMU

Parameter (Treatment time)	Wild-type	*sodB*
F_v/F_m in state 2 (0 hr)	0.27 ± 0.04	0.33 ± 0.05
F_v/F_m in state 2 (24 hr)	0.21 ± 0.02	0.18 ± 0.04
F_v/F_m in state 1 (0 hr)	0.49 ± 0.03	0.49 ± 0.02
F_v/F_m in state 1 (24 hr)	0.35 ± 0.03	0.17 ± 0.04
$F_m1 - F_m2/F_m2$ (0 hr)	0.96 ± 0.13	0.69 ± 0.14
$F_m1 - F_m2/F_m2$ (24 hr)	0.70 ± 0.08	−0.03 ± 0.05

Values are means ± SDs of the sample; n = 5 and 7 for wild-type and *sodB*, respectively. F_v, variable fluorescence; F_m, maximum fluorescence; F_m1, maximum fluorescence in state 1; F_m2, maximum fluorescence in state 2. $F_m1 - F_m2/F_m2$ quantifies the extent of the state 2-to-1 transition.
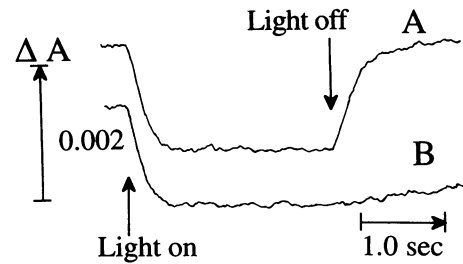

FIG. 2. Flash-induced absorbance change (ΔA) at 705 nm in intact cells of *sodB* mutant before (trace A) and after (trace B) 4 hr of treatment in 50 μM methyl viologen, elevated O_2, and 10 μmol·m^{-2}·s^{-1} orange light (633 nm). Measurements were made in 25 μM DCMU. Traces are averages of eight flashes. The dark interval between flashes was increased in trace B to allow full dark reversal of ΔA. The 705-nm ΔA in wild-type cells was identical to the *sodB* mutant above, except that the rate of dark reversal was less affected by methyl viologen treatment.

approximately linear increase in absorption after the actinic flash was calculated to represent the rate of reduction of P700 in darkness by reduced pools of cytochrome *c*553, cytochrome *f*, and other native electron donors to P700. These measurements were made in the presence of 25 μM DCMU, which abolishes electron flow from PSII. The effects of O_2 treatment in weak light on the characteristic features of the 705-nm absorbance change is summarized in Table 3. In the wild-type strain, only the rate of dark reduction of P700 decreased with treatment. In the *sodB* mutant, the dark reduction rate decreased more strongly than in the wild-type strain, and the oxidation rate and quantity of photooxidizable P700 also decreased.

The above experiments were also done by adding 50 μM methyl viologen (Table 4). The weak light in these treatments was changed to 10 μmol·m^{-2}·s^{-1} orange light (633 nm, 12-nm half-bandwidth), and the duration of the treatment was decreased to 4 hr. Although rates of dark reduction decreased in both strains, a more significant decrease was observed in the *sodB* mutant. The quantity of oxidizable P700 was unchanged in both strains, and the rate of oxidation was increased to a similar extent in both strains due to a decreased rate of simultaneous reduction.

Photoinhibition of PSII in the *sodB* Mutant. Photoinhibition of PSII by strong light has been proposed to occur by several hypothetical mechanisms, some of which include a role for O_2^- (17, 18). To test whether FeSOD has a role in protecting PSII from photoinhibition, samples of the wild-type and *sodB* strains were subjected to 600 μmol·m^{-2}·s^{-1} of narrow-band orange light (627 nm, 12-nm half-bandwidth) in chloramphenicol at 100 μg/ml and were sparged with 5% CO_2 in air during treatment. In intact cells, these conditions are known to cause rapid inactivation and degradation of the D1 protein of PSII (photoinhibition) while simultaneously abolishing the ability of the cell to synthesize replacement D1 protein (19, 20). The light-limited rate of oxygen evolution by the samples was measured at intervals during this treatment (Fig. 3). The extent and rate of photoinhibition exhibited by both strains

Table 3. Effects of elevated O_2 and 17 μmol m^{-2}·s^{-1} white light on the flash-induced absorbance transient at 705 nm in 25 μM DCMU

| Parameter | Fraction of control value after 24 hr | |
	Wild-type	*sodB*
Rate of *A* decrease	1.10 ± 0.16	0.42 ± 0.12
Extent of *A* change	1.07 ± 0.13	0.70 ± 0.16
Rate of *A* increase	0.44 ± 0.14	0.12 ± 0.06

Values are means ± SDs of the sample; n = 6 for all samples.

Table 4. Effects of 50 μM methyl viologen, elevated O_2, and 10 μmol·m^{-2}·s^{-1} orange light (633 nm) on the flash-induced absorbance transient at 705 nm

	Fraction of control value after 4 hr	
Parameter	Wild-type	*sodB*
Rate of *A* decrease	1.46 ± 0.31	1.47 ± 0.78
Extent of *A* change	1.11 ± 0.11	1.02 ± 0.14
Rate of *A* increase	0.24 ± 0.08	0.07 ± 0.03

Values are means ± SDs of the sample; $n = 6$ for all samples.

did not significantly differ, suggesting that FeSOD plays no role in protecting PSII from photoinhibition by strong light.

DISCUSSION

In this study we demonstrate the removal of FeSOD from an oxygen-evolving photosynthetic organism that remains viable when provided with supplemental inorganic carbon. Enzyme-activity assays indicate that the *sodB* mutant does not have significant FeSOD activity in its soluble protein fraction. MnSOD activity is present, however, in the membrane fraction of both wild-type and *sodB* strains. The presence of low levels of MnSOD in the thylakoid membranes of several cyanobacterial species, including *Anacystis nidulans*, has been demonstrated immunologically (5), and the enzyme has been purified to homogeneity from *P. boryanum* (16). Unlike other cyanobacterial MnSOD enzymes, the *A. nidulans* MnSOD is only partially inactivated by H_2O_2 (5). This observation would explain the presence of small amounts of H_2O_2-sensitive SOD in the membrane fraction of both the wild-type and *sodB* strains of *Synechococcus* sp. PCC 7942.

The effect of O_2 treatment and weak light on the chlorophyll fluorescence induction transient of the *sodB* mutant and wild-type strains demonstrates some FeSOD-dependent protection of PSII from damage by active oxygen under these conditions. Although variable fluorescence in fluorescence state 2 decreased in both strains with oxygen treatment, the decrease was more pronounced in the *sodB* mutant. Photoreduction of O_2 to O_2^- is not believed to occur at PSII (3), but because PSI and PSII are adjacent in the thylakoids of cyanobacteria, the O_2^- formed at PSI may contribute to the inactivation of PSII. Alternatively, greater depression of variable fluorescence by oxygen treatment in weak light could result from increased formation of singlet oxygen in the chlorophyll antenna of PSII. Formation of singlet oxygen in
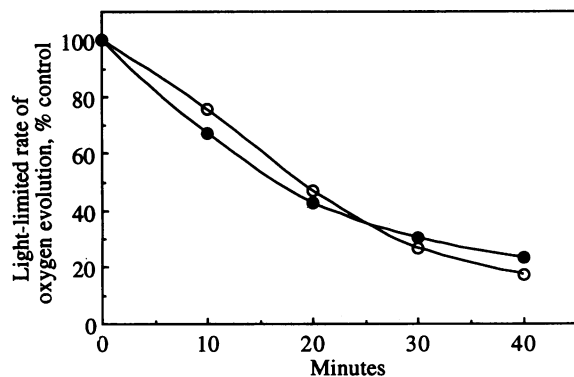

FIG. 3. Decline of light-limited rate of O_2 evolution with photo-inhibitory treatment in wild-type (o) and *sodB* (•) strains of *Synechococcus* sp PCC 7942. Treatment is described in *Results*. Measuring light was 15 μmol·m^{-2}·s^{-1} at 627 nm (12-nm half-bandwidth). Values are means of 8 and 10 samples for *sodB* and wild-type, respectively. SDs ranged from 2.86% to 13.1%.

both PSI and PSII could be promoted by damage elsewhere in the electron transport system, such damage being greater in the *sodB* strain, owing to the absence of FeSOD.

The effect of oxygen and weak light on variable fluorescence in fluorescence state 1 was substantially greater in the *sodB* mutant than in the wild-type strain. This result is, at least partially, due to the complete disappearance of the state 2-to-1 transition in the *sodB* mutant. In cyanobacteria, the state 2-to-1 transition is driven either by cyclic electron flow around PSI (15) or by oxidation of intersystem electron carriers by PSI (21). In either case, loss of the state transition indicates inactivation of electron transport through PSI. Photoacoustic measurements of energy storage by photosynthetic electron flow through PSI confirmed that O_2 sparging in weak light decreased PSI activity in the *sodB* mutant, whereas this treatment had no clear effect on the wild-type strain. This sensitivity of PSI to O_2 is to be expected in the *sodB* mutant because PSI is the primary site of O_2^- formation.

Oxygen-sensitivity of PSI in the *sodB* mutant is seen more specifically in the light-induced absorbance transient at 705 nm, which is indicative of the oxidation and reduction of PSI. These measurements indicated that treatment of the wild-type strain with O_2 and weak light affected only the rate at which P700 was reduced in darkness after the actinic flash. Conversely, the *sodB* mutant showed decreases in not only the dark reduction rate but also in the oxidation rate of P700 and in the quantity of oxidizable P700. Additionally, the decrease in the dark reduction rate of the *sodB* mutant was significantly greater than that observed in the wild-type strain. As was discussed for PSII, these effects on PSI could occur by direct action of O_2^-, via formation of hydroxyl radicals, on PSI components. Alternatively, they could also occur indirectly by the increased formation of singlet oxygen in the PSI antenna due to the inactivation of electron acceptors of PSI by hydroxyl radicals. These two possibilities were distinguished by the addition of methyl viologen to the treatment, which catalyzes O_2 photoreduction while minimizing formation of singlet oxygen by acting as an electron acceptor for PSI. Treatment with methyl viologen markedly decreased the rate of dark reduction in both strains, but the reduction was greater in the *sodB* mutant. Conversely, the methyl viologen treatment had no negative effect on the quantity of oxidizable P700 or on the oxidation rate in either strain. These results indicated that the negative effect of elevated O_2 (without methyl viologen treatment) on the quantity of oxidizable P700 and on the rate of its oxidation in the *sodB* mutant was from the destructive effect of singlet oxygen on the antenna and reaction center of PSI. The decreased dark reduction rate in both strains, however, was the direct effect of hydroxyl radicals on electron transport.

The absorbance transient at 705 nm was measured under conditions that abolished electron flow from PSII. Reduction of PSI after the actinic flash was therefore assumed to occur primarily by cyclic electron flow between PSI and the plastoquinone pool. Decreased rates of dark reduction after oxygen treatments in both strains were taken to indicate O_2^--mediated damage to some component of the cyclic pathway. On the donor side of PSI, oxygen treatment with methyl viologen slowed the dark reduction of the cytochrome *f*/cytochrome *c553* pool nearly as much as the dark reduction of P700 (our unpublished data), indicating that cyclic electron flow from cytochrome *f* to P700 was not strongly affected. On the accepting side of P700, electron flow to the iron–sulfur proteins that are the terminal electron acceptors of PSI, designated F_A and F_B, also appears intact, based on the slow increase of 705-nm absorbance after the actinic flash. Directed mutagenesis of F_A and F_B from PSI in the cyanobacterium *Anabaena variabilis* speeds up, rather than slows down, the postflash absorbance increase at 705 nm (22), owing to a much greater incidence of charge recombination

at P700. Therefore, in the present study, the component damaged by oxygen treatment appears to be in the return part of cyclic electron flow around PSI, between the iron–sulfur proteins of PSI and cytochrome f, most probably in the cytosol where FeSOD is present in the wild-type strain (Table 1) (7). Ferredoxin is a possible damage site because it not only reduces O_2 to O_2^-, but its iron can catalyze the formation of hydroxyl radicals via the Haber–Weiss reaction (3).

The presence of MnSOD in the thylakoids of both wild-type and *sodB* strains affects the interpretation of data presented in Fig. 3, in that O_2^- cannot be ruled out as a possible agent of damage to PSII by strong light. Equal sensitivity to photoinhibition of PSII by strong light in the two strains indicates that FeSOD does not protect against such photoinhibition. The possibility remains, however, that O_2^- generated within the thylakoid membrane may be the agent of damage because O_2^- in the thylakoids would be quenched equally by MnSOD in both strains.

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