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Iron Superoxide Dismutase Protects against Chilling Damage in the Cyanobacterium *Synechococcus* species PCC7942[1]

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A strain of *Synechococcus* sp. PCC7942 lacking functional Fe superoxide dismutase (SOD), designated *sodB*$^-$, was characterized by its growth rate, photosynthetic pigments, inhibition of photosynthetic electron transport activity, and total SOD activity at 0°C, 10°C, 17°C, and 27°C in moderate light. At 27°C, the *sodB*$^-$ and wild-type strains had similar growth rates, chlorophyll and carotenoid contents, and cyclic photosynthetic electron transport activity. The *sodB*$^-$ strain was more sensitive to chilling stress at 17°C than the wild type, indicating a role for FeSOD in protection against photooxidative damage during moderate chilling in light. However, both the wild-type and *sodB*$^-$ strains exhibited similar chilling damage at 0°C and 10°C, indicating that the FeSOD does not provide protection against severe chilling stress in light. Total SOD activity was lower in the *sodB*$^-$ strain than in the wild type at 17°C and 27°C. Total SOD activity decreased with decreasing temperature in both strains but more so in the wild type. Total SOD activity was equal in the two strains when assayed at 0°C.

Chilling susceptibility has been studied extensively in the cyanobacterium *Synechococcus* sp. strain PCC7942 (formerly *Anacystis nidulans* R2, hereafter referred to as PCC7942). Chilling stress has been shown to decrease cell viability (Siva et al., 1977), PET (Janz and Maclean, 1973; Ono and Murata, 1981a), and N_2 assimilation (Sakamoto and Bryant, 1998). Most studies of chilling stress in cyanobacteria have identified decreases in membrane fluidity as the primary cause of chilling injury (Ono and Murata, 1981a, 1981b, 1982; Murata and Wada, 1995; Somerville, 1995; Nishida and Murata, 1996). In support of this hypothesis, genetic manipulation of desaturase genes in cyanobacteria showed that increased desaturation of membrane lipids, which lowers the freezing point of the membranes, confers tolerance of chilling stress (Wada et al., 1990, 1994), whereas decreased desaturation of membrane lipids causes chilling sensitivity (Gombos et al., 1992; Tasaka et al., 1996; Sakamoto et al., 1998). It has been proposed that decreased membrane fluidity during chilling injures cyanobacteria by inhibiting the turnover of D1 protein that is required for photoinhibition repair at PSII (Gombos et al., 1994) and by inhibiting nitrate uptake and assimilation (Sakamoto and Bryant, 1998). Some investigators have also proposed that decreased membrane fluidity causes chilling injury in plants in the same ways that it does in cyanobacteria (Murata et al., 1992; Wolter et al., 1992; Miquel et al., 1993; Schneider et al., 1995; Wu et al., 1997).

In addition to direct effects on membranes, chilling in light imposes oxidative stress and increases the production of reactive forms of O_2, including the superoxide anion (O_2^-), in photosynthetic organisms (for review, see Wise, 1995). Low temperatures are proposed to decrease the activity of the Calvin cycle and other soluble enzymes without causing a concomitant decrease in light harvesting and PET. The excess reducing power created by light under these conditions generates reactive species of O_2. Production of H_2O_2 from O_2^- further inhibits specific enzymes of the Calvin cycle (Kaiser, 1979), and oxidative damage to photosystems ensues. Both chilling and moderate light are required for oxidative damage of this type. In the green alga *Chlamydomonas reinhardtii*, chilling combined with strong light caused rapid, irreversible damage to both PSI and PSII, whereas strong light at optimal temperature or chilling in darkness caused no lasting damage to either photosystem (Martin et al., 1997). Hodgson and Raison (1991) observed increased O_2^- production in isolated plant thylakoids during chilling treatment. In the green alga *Chlorella ellipsoidea*, Clare et al. (1984) identified O_2^- as an agent of chilling injury; the amount of SOD activity correlated with chilling tolerance. PSI has been identified as a primary target for oxidative damage during chilling in weak light in chilling-sensitive plants (for review, see Sonoike, 1996) and in chilling-tolerant plants (Tjus et al., 1998). We have observed similar inhibition of PSI in oxidatively stressed PCC7942 (Thomas et al., 1998). Thus, chilling stress in *Synechococcus* may include both oxidative stress and changes in membrane fluidity.

Studies of higher plants suggest a role for antioxidants in protection against chilling damage in photosynthetic cells. Increased expression of antioxidants occurs in several photosynthetic organisms during chilling stress. In wild tobacco, mRNA levels for the chloroplastic FeSOD increased moderately during chilling stress (Hérouart et al., 1991). Another study of wild tobacco showed that transcripts for MnSOD and Cu/ZnSOD increased during chilling stress as well (Tsang et al., 1991). Fryer et al. (1998) showed that the levels of both enzymatic and nonenzymatic antioxidants

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Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; NBT, nitroblue tetrazolium; NF, norflurazon; PET, photosynthetic electron transport; SOD, superoxide dismutase.

increased in maize under chilling conditions. There have been several attempts to make plants more tolerant of chilling stress by the expression of antioxidant transgenes. In some cases, overexpression of SODs resulted in plants that were indeed more tolerant to oxidative stress (Bowler et al., 1991; Perl et al., 1993; Sen Gupta et al., 1993a). Overexpression of pea Cu/ZnSOD in tobacco resulted in increased retention of photosynthetic activity at 3°C in 1500 μmol photons m^{-2} s^{-1} illumination, as compared with wild-type plants (Sen Gupta et al., 1993a, 1993b). Although these findings indicate a role for SODs in preventing damage from chilling, the same studies showed that the expression of foreign SODs in plants decreased the expression of native antioxidant genes. Total Cu/ZnSOD activity was higher in the transgenic plants than in the wild type, but expression of the native Cu/ZnSOD was suppressed. Ascorbate peroxidase activity was also increased in the transgenic SOD plants, casting some doubt on the role of SOD in chilling tolerance. The authors suggested that it was the combination of increased SOD and ascorbate peroxidase activity that conferred the tolerance to chilling.

To test the hypothesis that SODs are involved in protection against chilling damage in cyanobacteria, we compared the growth rate, SOD activity, photosynthetic pigments, and PSI activity of wild-type *Synechococcus* PCC7942 with a mutant of the same strain that is deficient in FeSOD. PCC7942 makes an excellent candidate for the study of antioxidants because it has a relatively simple antioxidant system consisting of a single catalase, a cytosolic FeSOD, thylakoid-associated MnSOD, and carotenoids (mostly β-carotene and zeaxanthin) associated with the photosynthetic apparatus (Laudenbach et al., 1989; Mutsuda et al., 1996; Thomas et al., 1998). PCC7942 lacks several of the antioxidants present in plants, including ascorbate peroxidase, α-tocopherol, and presumably glutathione peroxidase (Powls and Redfearn, 1967; Takeda et al., 1994). The *sodB*$^-$ strain of PCC7942, which lacks FeSOD, has been described previously (Laudenbach et al., 1989; Herbert et al., 1992; Samson et al., 1994; Thomas et al., 1998). The *sodB*$^-$ strain was sensitive to O_2^- generated in the cytosol, as indicated by decreased growth rate and PSI activity in the presence of methyl viologen. We predicted that, if cytosolic O_2^- contributes to chilling damage, the *sodB*$^-$ mutant would also be sensitized to chilling in the light. Our results show that the *sodB*$^-$ strain of PCC7942 is indeed sensitized to light-induced damage in the temperature range of 10°C to 17°C, but it is not more sensitive than the wild type at 0°C. This pattern appears to be determined by temperature effects on SOD activity.

MATERIALS AND METHODS

Culture and Experimental Conditions

Stock cultures of wild-type and *sodB*$^-$ PCC7942 were grown in 50-mL tubes of BG-11 medium (Sigma) supplemented with 10 mM $NaHCO_3$ and adjusted to pH 8.0 with 5 mM KH_2PO_4. Cultures were incubated in a water bath at 27°C, sparged with 3% CO_2 in air, and illuminated with cool-white fluorescent tubes at 15 to 35 μmol photons m^{-2} s^{-1} PAR. An LI-189 quantum sensor (Li-Cor, Lincoln, NE) measured light intensity. Light scattering, measured as apparent A_{750}, was used to monitor culture growth. Unless otherwise noted, cultures were diluted to A_{750} = 0.3 with fresh BG-11 medium without supplemental $NaHCO_3$ prior to experiments. Chilling-stress treatments were imposed in water-jacketed beakers maintained at 0°C, 10°C, 17°C, or 27°C in air and illuminated with 100 μmol photons m^{-2} s^{-1} from cool-white fluorescent tubes. Samples were stirred continuously during treatments.

Growth Measurements

Rapidly growing cells were centrifuged and resuspended in fresh BG-11 medium to an A_{750} of 0.1 and illuminated at 30 μmol photons m^{-2} s^{-1}. Cultures were sparged with 3% CO_2 in air as described above. Growth was measured at 10°C, 17°C, and 27°C. We removed 3-mL aliquots at 24- to 48-h intervals and measured A_{750}. A_{750} is strongly correlated with dry mass in PCC7942 over a wide range of cell sizes and stressful growth conditions (Thomas et al., 1998).

PSI Activity

We measured the photooxidation and dark reduction kinetics of P700 in intact cells using the broad band absorbance change centered at 820 nm (ΔA_{820}) as described elsewhere (Herbert et al., 1995; Thomas et al., 1998). The ΔA_{820} was monitored by reflectance using a modulated detection system consisting of a PAM 101 control unit and an ED 800T emitter-detector unit (Walz, Effeltrich, Germany). A branched fiber-optic cable delivered modulated 820-nm and white actinic light to the sample and collected the reflected 820-nm light. A tungsten projector lamp (model EJV, General Electric) fitted with three Calflex C heat filters (Balzers, Hudson, NH) and a mechanical shutter (Uniblitz VS25, Vincent Associates, Rochester, NY) provided actinic light (1000 μmol photons m^{-2} s^{-1}). Output from the control unit was collected and analyzed with a MacLab/2e data acquisition system using Scope version 3.3 software (AD Instruments, Milford, MA) on a Macintosh computer. We prepared samples for ΔA_{820} measurements by vacuum filtering 10 mL of culture at room temperature onto 0.45-μm membrane filters (type HA, Millipore). We then placed the filter and sample under an acrylic light guide at the end of the branched fiber-optic cable. We added electron transport inhibitors (DCMU and/or DBMIB) to the samples before filtration.

A_{820} transients were analyzed and interpreted as described previously (Yu et al., 1993) with the exception that we measured the initial slopes instead of using half-times to determine rates of oxidation and re-reduction of P700 (Thomas et al., 1998). We used inhibitors of electron transport to block different inputs of electrons to PSI as has been done previously (Maxwell and Biggins, 1976; Herbert et al., 1992; Yu et al., 1993; Thomas et al., 1998). Input from PSII was abolished with 25 μM DCMU. Input from the plastoquinone pool was blocked with 25 μM DBMIB. We made all measurements of PSI at room temperature.

Pigment Measurements

We used a DW-2000 scanning spectrophotometer (SLM/Aminco, Urbana, IL) to gauge photosynthetic pigments. We measured A_{625} and A_{678} in whole-cell suspensions and applied the calculations of Myers et al. (1980) to estimate phycocyanin concentrations. Chlorophyll and carotenoids were then extracted in 100% acetone. Chlorophyll *a* was quantified from A_{663} using an absorption coefficient of 88.2 mg chlorophyll *a* mL^{-1} cm^{-1}. The ratio of total carotenoids to chlorophyll *a* in the extract was estimated by comparing the integrated absorbance of the regions of the spectrum from 400 to 520 nm and from 640 to 690 nm. We used purified chlorophyll *a* from *Anacystis nidulans* (Sigma) as a reference for this ratio.

SOD Assay

Cells were pelleted by centrifugation at 1800*g* for 20 min and were resuspended in 50 mM K_2HPO_4 buffer, pH 7.8, with 0.5 mM EDTA. Suspensions were passed through a French press cell twice at 112 MPa to rupture the cells. Total SOD activity was measured in the resulting cell homogenates using the NBT photochemical assay (Beyer and Fridovich, 1987). A water-jacketed cuvette was placed in the DW-2000 spectrophotometer for the assay. A circulating water bath controlled the temperature of the cuvette. Reaction light was provided by a tungsten bulb (type EJA) fitted with two Calflex-C, a DT-Cyan, and two DT-Blue filters (Balzers). A fiber-optic bundle provided side-light to the cuvette in the spectrophotometer with an intensity of 500 μmol photons m^{-2} s^{-1}. A_{560} was measured for 60 s of reaction time. We placed a DT-Green and a DT-Yellow filter (Balzers) between the cuvette and the detector to prevent scattered reaction light from interfering with absorbance measurements. The first 20 s of the reaction were used to compute the initial rate of NBT to formazan conversion. We used the equation of Asada et al. (1974) to calculate the inhibition of this reaction by SOD. To isolate MnSOD activity, 50 μL of 3% H_2O_2 and 90 μL of 30 mM KCN were added to some of the 27°C samples. The H_2O_2 inhibited FeSOD and potassium cyanide prevented catalases from consuming the H_2O_2 (for review, see Bannister et al., 1987).

Analysis

We performed the data reduction and numerical analyses using Quattro Pro (Corel, Ottawa, Ontario, Canada) and tested differences between the samples for significance with the Student's *t* test (α = 5%). We replicated all of the experiments at least three times, using different starting cultures.

RESULTS

Growth

Both the wild-type and the *sodB*$^-$ strains exhibited similar growth patterns at 10°C, 17°C, and 27°C with 30 μmol photons m^{-2} s^{-1}. No growth occurred at 10°C or 17°C (Fig. 1). At 27°C normal growth occurred with similar growth rates in both strains. Based on the 10°C and 17°C data, we assumed that growth did not occur at 0°C. We also monitored growth during the chilling treatments and observed growth in both strains at 17°C and 27°C (Table I). As with the low-light experiment, growth was similar between the two strains at 27°C. However, at 17°C, growth of the *sodB*$^-$ strain was significantly lower than the wild type.

Functional P700

The amount of photooxidizable P700 in stressed and control cells is shown in Figure 2. To ensure that P700 was fully oxidized when we measured the extent of P700 photooxidation, we used saturating actinic light and treated the samples with 25 μM DCMU and 25 μM DBMIB. At 0°C both strains lost P700 at equal rates with most of the loss occurring between 96 and 120 h. However, at 10°C, 17°C, and 27°C, we saw significant differences between the wild-type and *sodB*$^-$ strains; the *sodB*$^-$ strain showed greater loss of P700 activity. At 27°C the wild type exhibited an increase in photooxidizable P700 that is thought to be a photoadaptation to decreased CO_2 and increased light in the stress beakers (Thomas et al., 1998). This adaptation response appeared to be depressed in the *sodB*$^-$ strain.

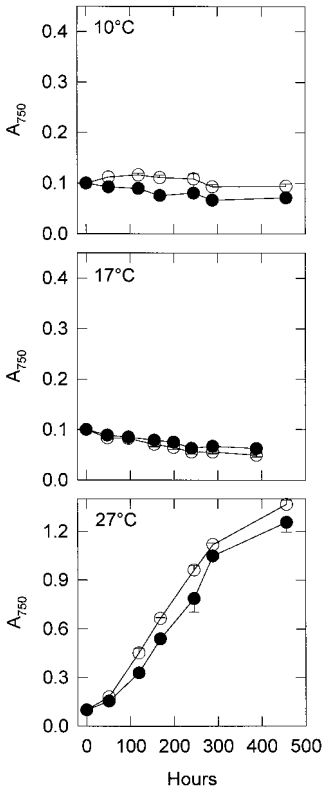

Figure 1. Growth rates of wild-type and *sodB*$^-$ strains. Growth curves measured at A_{750} are shown here for 10°C, 17°C, and 27°C. Each point is the mean of four to seven samples. Error bars are ±SD. Open symbols, Wild type; closed symbols, *sodB*$^-$.

Table I. *Changes in optical density during chilling and moderate light*

A_{750} was monitored as an indicator of growth during the P700 activity experiments. Cool-white fluorescent bulbs provided light with PAR flux of 100 μmol photons m^{-2} s^{-1}. Values are the mean percentages of starting values ± SDs after 120 h; n = 4 to 7 for each sample group. The only significant difference in growth/death between the wild-type and *sodB*$^-$ strains was seen at 17°C.

Strain	0°C	10°C	17°C	27°C
Wild type	76 ± 9.5	76 ± 6.7	183 ± 14.8	144 ± 10.8
sodB$^-$	83 ± 7.3	73 ± 2.6	113 ± 13.0	160 ± 26.7

P700 Oxidation Rate

The rates of P700 oxidation, which indicate the efficiency of light harvesting and excitation energy transfer to PSI, are shown in Figure 3. DBMIB (25 μM) was added to slow competing re-reduction of P700 and to ensure the maximum oxidation rate. At 27°C the *sodB*$^-$ strain had a relatively constant oxidation rate; it decreased slightly over a period of 48 h. At the same temperature, the wild type showed an initial increase in oxidation rate followed by a decrease to the same rate as the *sodB*$^-$ strain. This is also a photoadaptive response to decreased CO_2 and increased light in the wild type that is weaker in the *sodB*$^-$ strain (Thomas et al., 1998). At 17°C, both strains showed slight, but virtually identical, decreases in P700 oxidation. At 10°C the wild type exhibited the same decrease in P700 oxidation as at 17°C. However, the *sodB*$^-$ strain showed a much more pronounced decrease in P700 oxidation. At 0°C both strains had essentially the same response, with P700 oxidation dropping to less than 35% after 120 h.

P700 Re-Reduction Rate

The rate of P700 re-reduction (Fig. 4) was measured in the presence of 25 μM DCMU. This procedure is an indicator of PSI-driven cyclic electron transport (Yu et al., 1993; Herbert et al., 1995; Thomas et al., 1998). At 27°C both strains exhibited an initial increase in cyclic electron transport to approximately 170% of starting values, similar to previous observations (Thomas et al., 1998). A decrease to starting levels followed from 48 to 120 h. At 17°C we observed a dramatic difference between the two strains. The wild type increased cyclic activity to 200% of starting values over 120 h, whereas over the same period the *sodB*$^-$ strain decreased cyclic activity to less than 30% of starting values. At 0°C and 10°C both strains lost cyclic activity at essentially the same rate, with the loss occurring more rapidly at 0°C. At 0°C and 10°C in both strains, and at 17°C in the *sodB*$^-$ strain, cyclic PET decreased more rapidly than the amount of photooxidizable P700 or the rate of P700 oxidation.

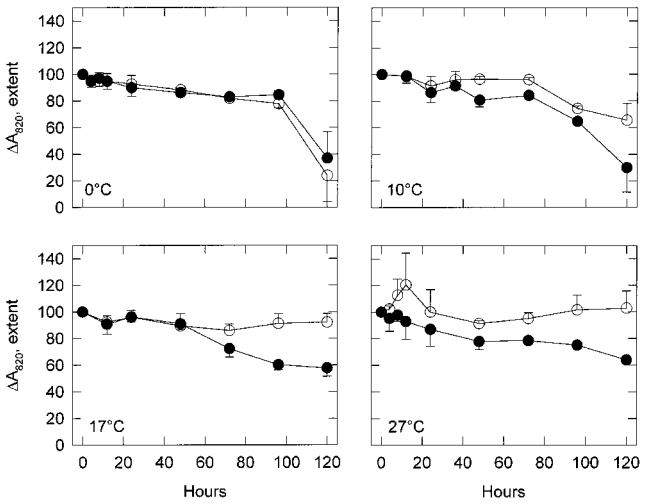

Figure 2. P700 oxidation extent. ΔA_{820} was used to measure the relative amount of photooxidizable P700. All data are percentages of starting values. Each point is the mean of four to eight samples. Error bars are ±SD. Open symbols, Wild type; closed symbols, *sodB*$^-$.

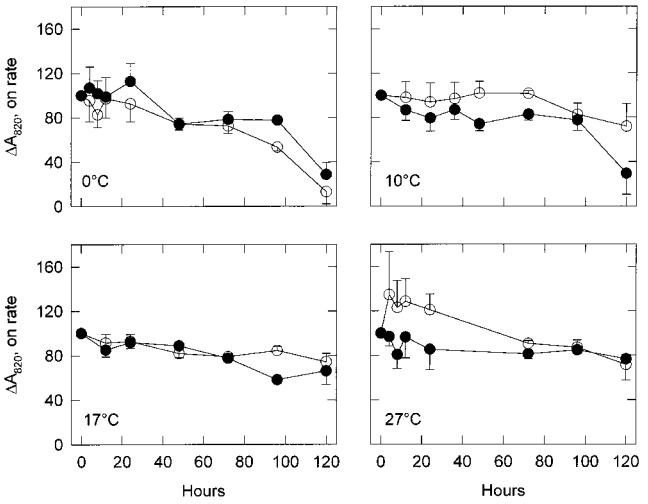

Figure 3. P700 oxidation rate. The ΔA_{820} over the initial 7.5 to 10 ms after the onset of the actinic flash was used to calculate the initial rate of P700 oxidation. Each point is the mean of four to eight samples and all data are percentages of initial values. Error bars are ±SD. DBMIB (25 μM) was added to all samples to prevent competing re-reduction by the cyclic and noncyclic pathways. Open symbols, Wild type; closed symbols, *sodB*$^-$.

Photosynthetic Pigments

With the exception of the 17°C treatment, phycocyanin concentrations were almost identical in both strains (Fig. 5). At 27°C phycocyanin concentrations increased slightly over 120 h. The 0°C and 10°C treatments resulted in the loss of phycocyanin. At 17°C the *sodB*$^-$ strain showed little change in phycocyanin content, whereas the phycocyanin concentration increased significantly in the wild type. Extractable chlorophyll data appear in Figure 6. At 27°C there were no significant differences in chlorophyll *a* content between the two strains, which remained constant throughout the experiments. We saw results in the 10°C treatment that were similar to the 27°C treatment, but the data were more variable. In the 17°C treatment, the wild type had approximately 50% more chlorophyll *a* than the *sodB*$^-$ strain after 120 h. At 0°C both strains lost chlorophyll

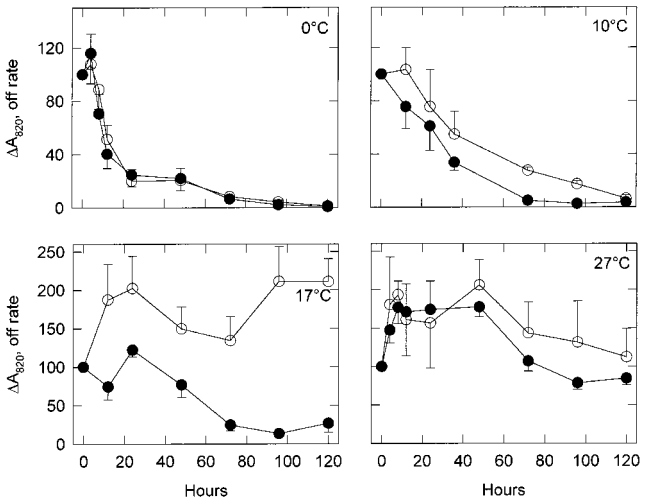

Figure 4. P700 re-reduction rate with DCMU. The ΔA_{820} over the initial 30 to 300 ms after the actinic flash was used to calculate the initial rate of P700 re-reduction. Each point is the mean of four to eight samples and all data are percentages of initial values. Error bars are ±SD. The addition of 25 μM DCMU blocked input from PSII. The data shown represent the rates of cyclic electron transport. Open symbols, Wild type; closed symbols, *sodB*$^-$.

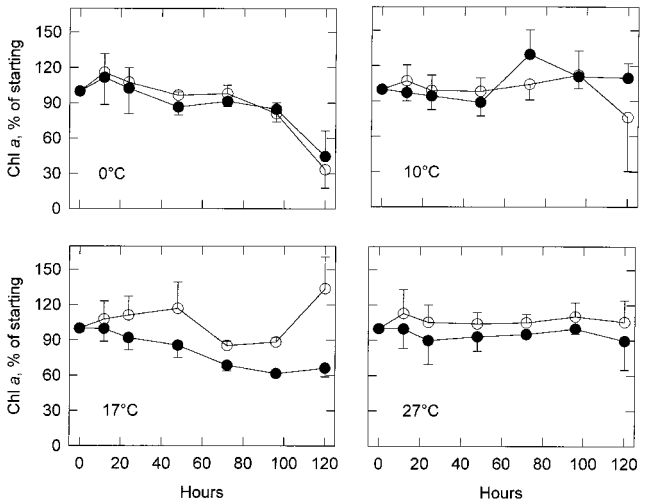

Figure 6. Spectrophotometric measurements of acetone-extractable chlorophyll *a*. Chlorophyll (Chl) was extracted into 100% acetone. Extractable chlorophyll concentrations are shown for wild type and *sodB*$^-$ as measured by A_{663}. Each point is the mean of four to eight samples. Error bars are ±SD. Open symbols, Wild type; closed symbols, *sod*$^-$.

at equal rates with only about 40% of the original chlorophyll concentration remaining at the end of 120 h. There was little difference between the two strains in the ratios of carotenoids to chlorophyll (Fig. 7). The ratio decreased slightly in the 0°C and 10°C treatments and remained constant or increased slightly in the 17°C and 27°C treatments. Comparison of Figures 5 and 6 shows that chlorophyll was lost more rapidly than phycocyanin during chilling stress.

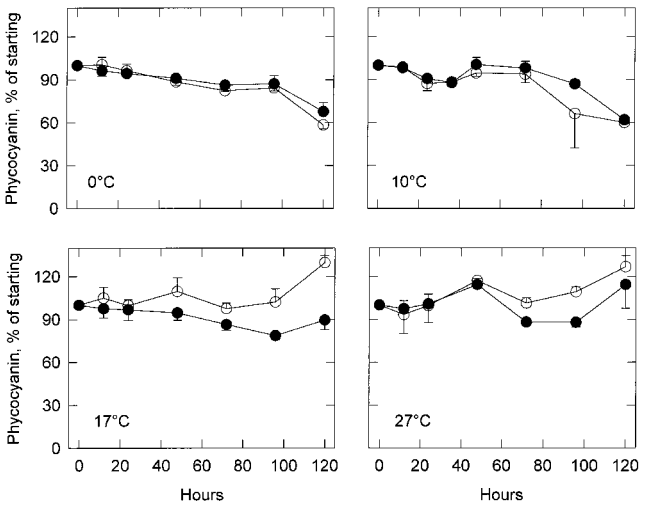

Figure 5. Spectrophotometric measurements of phycocyanin in whole cells. Phycocyanin concentrations are shown for wild-type and *sodB*$^-$ strains as measured by A_{625} and A_{678}. Each point is the mean of four to eight samples. Error bars are ±SD. Open symbols, Wild type; closed symbols, *sodB*$^-$.

SOD Activity

Total SOD activity in cell homogenates decreased with decreasing temperature (Fig. 8). These measurements were made in cells that had not been stressed to determine the direct effect of temperature on SOD function. As expected, the *sodB*$^-$ strain had much less total SOD activity than the wild type because of the lack of FeSOD. Total SOD activity declined more rapidly with temperature in the wild type

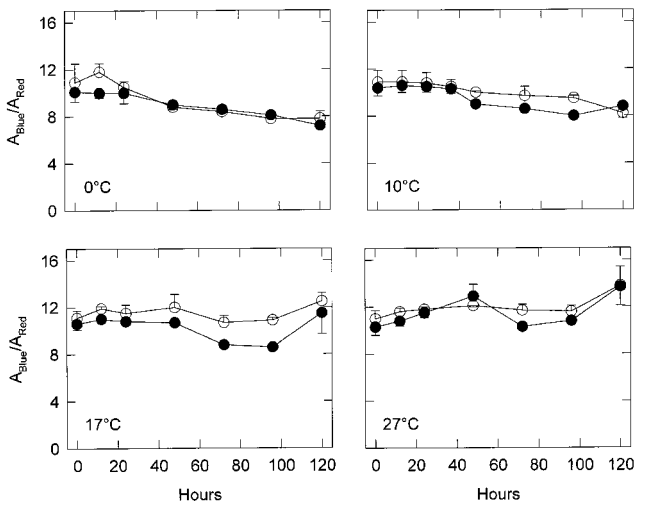

Figure 7. Carotenoid-to-chlorophyll ratios. Chlorophyll *a* and carotenoids were extracted into 100% acetone. The ratio of blue absorbance (400–520 nm) to red absorbance (650–690 nm) was calculated as a relative indicator of the carotenoid-to-chlorophyll *a* ratio. Each point is the mean of four to eight samples. Error bars are ±SD. For comparison, purified chlorophyll *a* from *A. nidulans* yielded a blue-to-red ratio of 4.53. Open symbols, Wild type; closed symbols, *sodB*$^-$.

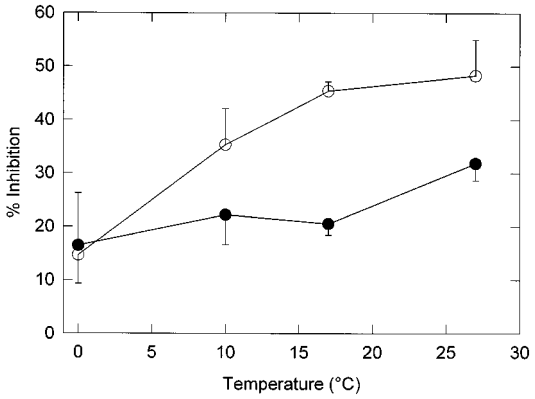

Figure 8. Total SOD activity in cell extracts. SOD activity was measured in crude cell extracts as inhibition of the NBT to formazan conversion. Data points are means of three to six samples; error bars are ±SD. Open symbols, Wild type; closed symbols, *sodB*$^-$.

than in the *sodB*$^-$ strain. We also found that the *sodB*$^-$ strain had more MnSOD activity than the wild type (data not shown), confirming earlier findings (Herbert et al., 1992).

DISCUSSION

Loss of cytosolic FeSOD activity in the *sodB*$^-$ mutant sensitized it to moderate chilling stress (10°C–17°C) in the presence of light. The loss of FeSOD activity appeared to make no difference in sensitivity to severe chilling stress (0°C–10°C) in light. The differences between the *sodB*$^-$ and the wild-type strains in cyclic PET activity, pigment content, and other parameters were much more pronounced at 17°C than at lower temperatures. Previous research on PCC7942 (Thomas et al., 1998) showed that the *sodB*$^-$ strain was more sensitive to paraquat-catalyzed O_2^- than was the wild type. However, the *sodB*$^-$ strain was less sensitive than the wild type to NF treatment, which stimulates production of excited singlet-state oxygen ($^1O_2$) in the photosynthetic antennae. It was hypothesized that increased MnSOD activity in the *sodB*$^-$ mutant conferred resistance to $^1O_2$. It is unlikely that MnSOD directly detoxifies $^1O_2$, but elevated MnSOD activity in the *sodB*$^-$ strain may protect the cells from $^1O_2$ indirectly. Organic radicals generated by $^1O_2$ may give rise to O_2^- (Asada and Takahashi, 1987; Scandalios, 1993), which would be quenched by the MnSOD.

The most chilling-sensitive parameter tested was the re-reduction of P700 by cyclic electron transport. P700 re-reduction rates declined almost immediately at 0°C and 10°C (Fig. 4), whereas loss of light harvesting efficiency and photooxidizable P700 occurred much later (Figs. 2 and 3). Decreases in the amount of chlorophyll *a* closely paralleled P700 loss (Fig. 6), and because carotenoid-to-chlorophyll ratios remained fairly constant (Fig. 7), the carotenoids declined at the same rate as chlorophyll. Phycocyanin was resistant to degradation with only about 35% lost after 120 h at 0°C (Fig. 5). The cultures were visibly colored blue after this treatment. This pattern of damage resembled the pattern observed when the two strains were treated with paraquat in light, which produces O_2^- on the reducing side of PSI (Thomas et al., 1998). The early inhibition of P700 re-reduction with loss of functional P700 occurring much later suggests oxidative damage to the Fe-S clusters of the F_A/F_B sites of PSI and/or Fd. These sites are damaged early during oxidative stress and are known to be disrupted by superoxide (Fujii et al., 1990; Dodge, 1991; Liochev, 1996). The damage caused by chilling in light is not consistent with an increase in $^1O_2$ production. If increased $^1O_2$ production occurred during chilling, we would expect the decrease in the rate and extent of P700 oxidation to occur earlier and to a greater extent, as was observed previously in cultures treated with NF (Thomas et al., 1998). In addition, if $^1O_2$ were an agent of chilling damage, we would expect the *sodB*$^-$ strain to show some resistance to chilling because this strain previously was moderately resistant to NF (Thomas et al., 1998).

Chilling temperatures stopped the growth of both the wild-type and *sodB*$^-$ strains of PCC7942 when grown under 30 μmol photons m^{-2} s^{-1} (Fig. 1). During stress treatments at 100 μmol photons m^{-2} s^{-1}, however, both strains exhibited growth at 17°C (Table I). The *sodB*$^-$ strain grew much slower than the wild type under these conditions, verifying that the damage to electron transport that we observed was relevant to growth and survival of the organism. The effect of stronger light overcoming chilling stress and allowing growth at 17°C in the wild type indicates that adaptation or repair processes that allow growth at low temperatures require energy from photosynthesis. The slower growth of the *sodB*$^-$ mutant at 17°C indicates either that chilling adaptation processes are inoperative or that rates of chilling damage approached or exceeded rates of repair.

Lack of the FeSOD in the *sodB*$^-$ strain of PCC7942 plainly sensitizes this mutant to chilling at 17°C (Table I; Fig. 3). At 0°C, however, the *sodB*$^-$ and wild-type strains are equally chilling sensitive. This pattern can be explained by low-temperature inactivation of SODs. The total SOD activity in the wild type is more than twice that of the *sodB*$^-$ strain at 17°C, whereas at 0°C, total SOD activity in the two strains is equal (Fig. 8). Greater total SOD activity in the wild type than in the *sodB*$^-$ strain at 27°C is consistent with previous measurements that showed the FeSOD fraction of total SOD activity to be absent from the *sodB*$^-$ strain (Herbert et al., 1992). The *sodB*$^-$ strain also showed a 50% increase in MnSOD activity that is probably a regulatory compensation for the absence of FeSOD.

The effect of temperature on total SOD activity was different in the *sodB*$^-$ and wild-type strains (Fig. 8). Total SOD activity in the wild type, which is a mixture of FeSOD and MnSOD activity, showed a typical Q_{10} value (increase in the processing rate produced by raising the temperature 10°C) of approximately 2 over the range of 0°C to 17°C and was stable from 17°C to 27°C. In contrast, total SOD activity in the *sodB*$^-$ strain, which is entirely MnSOD activity, showed a Q_{10} of approximately 1.3. This difference is unexpected because the purified FeSODs and MnSODs of many organisms have very similar structures and operate at diffusion-limited rates (Fridovich, 1989). However, the MnSOD in PCC7942 is membrane bound (Herbert et al.,

1992), and its apparent temperature insensitivity may stem from this circumstance. In the NBT assay O_2^- is generated by the illumination of riboflavin in the reaction solution, and SOD activity is determined from inhibition of the superoxide-dependent conversion of NBT to a blue-colored formazan. In the cell homogenates that we used for the SOD assays, O_2^- formation by PSI activity was also likely. If the membrane-bound MnSOD of PCC7942 were closely associated with the O_2^--generating site of PSI, its activity could appear less temperature sensitive than that of the soluble FeSOD. Supporting this hypothesis, Asada et al. (1998) proposed a close physical association of CuZnSOD with PSI in chloroplasts.

Nutrient deficiencies may mask symptoms of photooxidative stress during chilling of cyanobacteria. Recent research on nitrate assimilation (Sakamoto and Bryant, 1998) showed that chilling inhibits the uptake of nitrate in *Synechococcus* sp. PCC7002, resulting in a visible chlorotic condition. Similar though less severe chlorosis was observed in *Synechococcus* sp. PCC6301 and *Synechocystis* sp. PCC6803. At no time during our experiments did we observe similar chlorosis in either strain of PCC7942. The high phycocyanin-to-chlorophyll *a* ratio seen in Figures 5 and 6 is the opposite of pigment changes caused by N_2 starvation (Collier et al., 1994). Because BG-11 contains ferric ammonium citrate, the cyanobacteria in our experiments had a source of nitrogen other than nitrate.

In addition to membrane lipid desaturation and antioxidant activity, other factors may contribute to chilling tolerance in cyanobacteria. Genetically increasing the concentration of Gly-betaine in PCC7942 conferred tolerance to both chilling and salt stress (Deshnium et al., 1995, 1997). The high Gly-betaine transformants in this study also showed an in vivo phase transition of membrane lipids at lower temperatures than did the wild-type controls, although this effect could not be replicated in vitro. Heat-shock proteins also moderated chilling injury in cyanobacteria. Inactivation of genes for the ClpB chaperone and the P-subunit of the Clp protease sensitized PCC7942 to moderate chilling stress (Porankiewicz and Clarke, 1997; Porankiewicz et al., 1998). Heat-shock proteins also increased chilling tolerance in plants (Sabehat et al., 1998).

Chilling tolerance is a goal of agricultural genetic engineering. Our results indicate that, although SODs may have a role in chilling resistance, they are themselves inactivated during severe chilling stress. At lower temperatures, nonenzymatic antioxidants such as β-carotene and α-tocopherol may play greater roles in cyanobacteria and in plants, making them targets for genetic manipulation. Alternatively, transgenes for antioxidant enzymes from psychrophilic organisms that retain their activity at 0°C and below might be effective in conferring chilling tolerance. Integrated manipulation of membrane lipid saturation, enzymatic and nonenzymatic antioxidants, and other factors may be necessary for meaningful improvements in chilling tolerance. Cyanobacteria provide a good model for testing these strategies.

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