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Photoinhibition and Light-Induced Cyclic Electron Transport in *ndhB*⁻ and *psaE*⁻ Mutants of *Synechocystis* sp. PCC 6803

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The *ndhB*$^-$ and *psaE*$^-$ mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 are partly deficient in PSI-driven cyclic electron transport. We compared photoinhibition in these mutants to the wild type to test the hypothesis that PSI cyclic electron transport protects against photoinhibition. Photoinhibitory treatment greatly accelerated PSI cyclic electron transport in the wild type and also in both the mutants. The *psaE*$^-$ mutant showed rates of PSI cyclic electron transport similar to the wild type under all conditions tested. The *ndhB*$^-$ mutant showed much lower rates of PSI cyclic electron transport than the wild type following brief dark adaptation but exceeded wild type rates after exposure to photoinhibitory light. The wild type and both mutants showed similar rates of photoinhibition damage and photoinhibition repair at PSII. Photoinhibition at PSI was much slower than at PSII and was also similar between the wild type and both mutants, despite the known instability of PSI in the *psaE*$^-$ mutant. We conclude that photoinhibitory light induces sufficient PSI-driven cyclic electron transport in both the *ndhB*$^-$ and *psaE*$^-$ mutants to fulfill any role that cyclic electron transport plays in protection against photoinhibition.

Key words: Cyanobacteria — *ndhB* — Photoinhibition — Photosynthesis — *psaE* — PSI cyclic electron transport.

Abbreviations: ΔS_{820}, the change in reflectance signal at 820 nm that results from photooxidation of P700; DMBQ, 2,6-dimethylbenzoquinone; FQR, the putative ferredoxin-quinone oxidoreductase; KFeCN, potassium ferricyanide; MV, methyl viologen (paraquat); NDH-1, the thylakoid NAD(P)H dehydrogenase; PAR, photosynthetically active radiation; PET, photosynthetic electron transport.

Introduction

Reactive oxygen species are normal byproducts of photosynthetic light-harvesting and electron transport. As a consequence, all oxygen-producing photosynthetic organisms must cope with the potential for photooxidative damage to PSII, PSI, and other components of the photosynthetic system (Powles 1984, Asada 1994, Wise 1995, Sonoike 1996, Martin et al. 1997, Asada et al. 1998, Melis 1999, Tjus et al. 1999). At normal temperatures, excessive irradiance causes a type of photooxidative damage to PSII known as photoinhibition. In intact cells, photoinhibition occurs when the Q_A acceptor of PSII is already reduced at the time of charge separation. Lack of oxidized Q_A promotes recombination between reduced pheophytin and P680$^+$ in PSII, yielding an excited triplet state of P680 that in turn generates excited singlet oxygen. Excited singlet oxygen oxidizes the D1 protein of PSII in a way that inactivates electron transport and also renders the D1 protein susceptible to proteolysis. Photoinhibition damage to PSII is rapidly repaired by partial disassembly of inactivated PSII complexes, lysis of the damaged D1 protein, de novo synthesis and replacement of D1, reassembly of PSII, and reactivation of electron transport through the repaired PSII complex. (Kyle et al. 1984, Durrant et al. 1990, Vass et al. 1992, Aro et al. 1993, Andersson and Barber 1996, Hideg et al. 1998, Melis 1999). Photoinhibition damage at PSII is favored under any conditions in which the excitation of P680 exceeds the capacity of photosynthetic electron transport (PET), which is in turn dependent on downstream metabolism such as CO_2 assimilation, NO_3^- assimilation, and other processes (Asada 1999, Niyogi 2000). For this reason, stress conditions that slow downstream metabolism concomitantly accelerate photoinhibition damage to PSII. Photoinhibition damage at PSII may be photoprotective because it slows the generation of reactive oxygen elsewhere in the photosynthetic system and thus prevents oxidative damage to other targets that are not as readily repaired as is PSII, such as PSI (Sonoike 1996, Asada 1999). Nonetheless, the hypothesis that photoinhibition damage to PSII is itself deleterious to the fitness of photosynthetic organisms is supported by the existence of photoprotective xanthophyll cycling and other adaptations that serve to minimize it (Yamamoto and Bassi 1996, Niyogi et al. 1998, Demmig-Adams and Adams 2000).

The cyclic paths of PET driven by PSI have been proposed to protect PSII from photoinhibition (Canaani et al. 1989, Topf et al. 1992, Heber and Walker 1992, Fork and Herbert 1993, Herbert et al. 1995, Endo et al. 1999). At least two PSI-driven electron transport cycles are known to occur in plants, algae, and cyanobacteria. The most familiar involves a putative ferredoxin-plastoquinone oxidoreductase (FQR) that

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transfers electrons from ferredoxin (Fd) to plastoquinone (PQ) (Bendall and Manasse 1995, Scheller 1996, Endo et al. 1997, Endo et al. 1998). The FQR cycle is completed as electrons move from PQ back to PSI via the cytochrome b_6f complex and plastocyanin. A longer PSI cycle involving a thylakoid NAD(P)H-plastoquinone oxidoreductase (NDH-1) is also known (Ogawa 1992, Mi et al. 1992, Yu et al. 1993, Ravenel et al. 1994, Mi et al. 1995, Shikanai et al. 1998). In the NDH-1 cycle, NADPH generated by linear PET is oxidized and PQ is reduced by NDH-1. From PQ to PSI the NDH-1 cycle is the same as the FQR pathway. The thylakoid NDH-1 is homologous to Complex I of respiratory electron transport except that it lacks several extrinsic subunits (Friedrich et al. 1995, Friedrich and Weiss 1997). In cyanobacteria, the thylakoid NDH-1 participates in respiratory electron transport as well as the PSI cycle described above (Hirano et al. 1980, Scherer 1990, Gantt 1994, Peschek 1996). In addition to oxidizing NADPH from PET, the thylakoid NDH-1 of cyanobacteria can oxidize NAD(P)H derived from respiratory pathways. For example, addition of glucose to *Synechocystis* sp. PCC 6803 increases the electron flux into PSI when input from PSII is blocked with DCMU (Hirano et al. 1980, Thomas and Herbert unpublished). The thylakoid NDH-1 of cyanobacteria is specific for NADPH (Matsuo et al. 1998) but input of NADH from glycolysis to the thylakoid NDH-1 could occur if NADH is converted to NADPH by a pyridine nucleotide transhydrogenase. There are putative sequences for such a transhydrogenase in the *Synechocystis* genome (*pntA* and *pntB*, Cyanobase database [www.kazusa.or.jp/cyano/cyano.html], Nakamura et al. 1998). Significant reduction of the plastoquinone pool by succinate dehydrogenases has also been shown to occur in *Synechocystis* PCC 6803 (Cooley et al. 2000).

There are several hypotheses for how the PSI cycles of PET may protect against photoinhibition. Heber and Walker (1992) have proposed that PSI cycles sustain a proton gradient when PSII and linear electron transport are inhibited or down-regulated by excessive light. The proton gradient in turn maintains photoprotective mechanisms in the PSII antenna that reduce the input of excitation to P680. This hypothesis is applicable to plants and green algae but cyanobacteria do not exhibit the xanthophyll-dependent photoprotective mechanisms present in chlorophytes (Campbell et al. 1998). Cyanobacteria do exhibit dramatic state transitions, however, which are dependent on the operation of PSI cyclic pathways (Satoh and Fork 1983, Herbert et al. 1992, Schreiber et al. 1995). State 1 to State 2 transitions redistribute excitation from PSII to PSI. This redistribution may be photoprotective since, at normal temperatures, PSI is less susceptible to photooxidative damage than PSII (Canaani et al. 1989, Havaux and Eyeletters 1991, Martin et al. 1997). PSI cycles may also protect against photoinhibition by increasing the ratio of ATP to NADPH that is generated by PET. Additional ATP from cyclic photophosphorylation may contribute to the repair of photoinhibited PSII in cyanobacteria. Synthesis of D1 protein requires approximately 350 mol ATP per mol protein. In eukaryotes, part of this ATP could come from cytosolic and mitochondrial sources via plastidic ATP/ADP translocators (Winkler and Neuhaus 1999). In cyanobacteria, which lack such external sources of ATP, PSI-driven cyclic photophosphorylation may be an important source of the ATP needed for photoinhibition repair, especially when PSII activity has been photoinhibited by strong light. The ATP generated by PSI cycles is also used to drive active uptake of CO_2 in cyanobacteria. Several NDH-1 mutants of *Synechocystis* have been shown to be impaired in CO_2 uptake (Ohkawa et al. 2000a, Ohkawa et al. 2000b, Klughammer et al. 1999). Uptake of CO_2, driven by PSI cycles, would tend to protect against photoinhibition by supporting high rates of CO_2 assimilation.

The hypothesis that PSI cyclic electron transport protects against photoinhibition in cyanobacteria can be tested using mutants defective in the PSI cycles. A variety of such mutants have been constructed in different strains of cyanobacteria. Insertional mutations of *psaE* have been made in *Synechocystis* sp. PCC 6803 and in *Synechococcus* sp. PCC 7002 (Chitnis et al. 1989, Zhao et al. 1993). In both cases, the loss of PsaE resulted in decreased PSI cyclic electron transport consistent with the participation of PsaE in the FQR-mediated PSI cycle (Zhao et al. 1993, Yu et al. 1993). Mutations in genes for the thylakoid NDH-1 also decrease PSI cyclic electron transport. Mutation of *ndhB* in *Synechocystis* sp. PCC 6803 virtually abolished cyclic PET, leading to the hypothesis that cyclic PET occurs only through the NDH-1 pathway in cyanobacteria (Mi et al. 1992). Mutation of an *ndhF* in *Synechococcus* sp. PCC 7002 resulted in only partial inhibition of the PSI cyclic paths, however, supporting the alternate hypothesis that PSI drives separate FQR and NDH-1 cycles (Yu et al. 1993, Bendall and Manasse 1995). A double mutant lacking both PsaE and NdhF in *Synechococcus* sp. PCC 7002 showed no PSI cyclic electron transport, also supporting the two path hypothesis (Yu et al. 1993).

The *ndhB*$^-$ and *psaE*$^-$ mutants of cyanobacteria provide a means to test the hypothesis that cyclic electron transport around PSI slows photoinhibition of PSII. In the present study, photoinhibition of PSII by strong light was tested in wild-type, *ndhB*$^-$, and *psaE*$^-$ strains of *Synechocystis* sp. PCC 6803. The rates of both photoinhibition damage and photoinhibition repair in the mutants were similar to the wild type. Photoinhibitory treatments induced a high rate of PSI cyclic electron transport in all strains, however, including the *ndhB*$^-$ mutant. Therefore our results do not disprove the hypothesis that PSI cycles protect against photoinhibition. Our results do support the hypotheses that both FQR- and NDH-mediated cycles are present in cyanobacteria and that they are stimulated by strong light.

Results

Growth and pigments

Growth of all strains was very similar under 3% CO_2 in air

Table 1 Growth and pigment analysis of *Synechocystis* sp. PCC 6803 wild type and mutant strains. Growth rates represent the maximum rates achieved after an initial lag in growth

Strain	Growth (ΔA_{750} $h^{-1} \times 10^4$)	Phycocyanin (mM)	Chl *a* (mM)	Total carotenoids/Chl *a* (relative units)
Wild type	45.0	32.5±3.0	17.8±7.5	9.26±0.07
ndhB⁻	50.7	33.2±1.2	15.7±2.2 [a]	11.1±0.08 [a]
psaE⁻	43.9	31.6±0.6	15.3±2.5 [a]	9.90±0.09 [a]

Pigment values are means of eight separate cultures±variances.
[a] Values are significantly different from wild type at $\alpha = 0.05$.

(Table 1). The phenotype of the *ndhB*⁻ mutant (Ogawa 1991, Ohkawa et al. 2000b) was confirmed by its inability to grow without addition of 3% CO_2 in air to the cultures (data not shown). The *psaE*⁻ mutant grew similarly to wild type under all conditions, as observed previously (Chitnis et al. 1989, Xu et al. 1994).

Pigment analysis revealed small but statistically significant pigment differences between strains (Table 1). Both mutant strains had significantly higher carotenoid to Chl ratios than the wild type. The *ndhB*⁻ mutant also exhibited a significantly higher ratio of phycocyanin to Chl than the other two strains.

The effect of strong light on PSII activity

The effect of strong light on PSII activity was measured in intact cells by their light-saturated gross O_2 evolution over a time course of exposure to 3,000 μmol photons m^{-2} s^{-1} PAR. There were no significant differences between wild type and mutants during any of these experiments (Fig. 1, 2). Without spectinomycin, photoinhibition of PSII was not observed when its activity was limited by downstream electron transport (Fig. 1A). With addition of DMBQ and KFeCN, however, PSII activity was uncoupled from downstream electron transport and photoinhibition of PSII was observed during the first 120 min of strong light treatment (Fig. 1B). During this time, PSII activity declined to the levels observed without DMBQ and KFeCN addition and then stabilized for the remaining 180 min of treatment. With addition of 75 μM spectinomycin, photoinhibition of PSII activity was accelerated and was observed even when limited by downstream electron transport (Fig. 2). Spectinomycin prevents de novo synthesis of D1 protein, allowing the rate of photoinhibition damage to be observed independently of photoinhibition repair (Samuelsson et al. 1985, Samuelsson et al. 1987, Herbert 1990, Krupa et al. 1990, Martin et al. 1997). The wild-type, *ndhB*⁻, and *psaE*⁻ strains exhibited similar rates of photoinhibition in the presence and the absence of spectinomycin, indicating similar rates of photoinhibition damage and photoinhibition repair in the three strains (Fig. 1, 2).

The effect of strong light on PSI activity

The quantity of photooxidizable P700 present at the start of photoinhibition treatments was very similar in the all three strains (Fig. 3A, 4A). The apparently small quantity of photooxidizable P700 in the *ndhB*⁻ mutant at time 0 was an artifact of incomplete dark reduction of $P700^+$ between the actinic flashes owing to the very low rate of $P700^+$ re-reduction before strong light treatment (data not shown). Photoinhibitory treatments in both the absence and the presence of spectinomycin caused only a small decrease in the quantity of photooxidizable P700 that was similar in the wild-type and mutant strains (Fig. 3A, 4A).

Rates of electron input to $P700^+$ by PSI-driven cyclic electron transport are shown in Fig. 3B and 4B. During these measurements, PSII activity was blocked with 25 μM DCMU, leaving the PSI-driven cyclic paths as the only source of electron input to PSI. Following the 20 min dark adaptation at the start of photoinhibition treatments, the rate of $P700^+$ reduction by the cyclic electron transport was similar in the wild-type and *psaE*⁻ strains but much slower in the *ndhB*⁻ strain. Photoinhibitory treatments caused a dramatic increase in the rate of PSI cyclic electron transport in all three strains. Most notably, the *ndhB*⁻ strain showed a PSI cyclic electron transport rate close to zero before photoinhibitory treatment but showed a significantly higher rate than either the *psaE*⁻ mutant or the wild type after 60 min of strong light. Over the subsequent 5 h of strong light exposure, the PSI cyclic electron transport rates in the *psaE*⁻ mutant and wild type continued to increase while rates in the *ndhB*⁻ mutant slowed so that all were similar at the end of the treatment. The PSI cyclic electron transport rates of all three strains increased during photoinhibitory treatments in the presence of 75 μM spectinomycin but less so than in its absence. Dark controls showed no direct effects of 75 μM spectinomycin on either P700 extent or PSI cyclic electron transport rate (data not shown).

The effect of methyl viologen on accelerated PSI cyclic electron transport

In a separate experiment, rates of PSI cyclic electron transport with and without addition of 100 μM MV were determined before and after exposure to 1,500 μmol photons m^{-2} s^{-1} (Fig. 5). At 100 μM, MV competitively inhibits the reduction of ferredoxin by PSI and thus inhibits both FQR- and NDH-dependent PSI cycles (Fujii et al. 1990, Yu et al. 1993). Data of

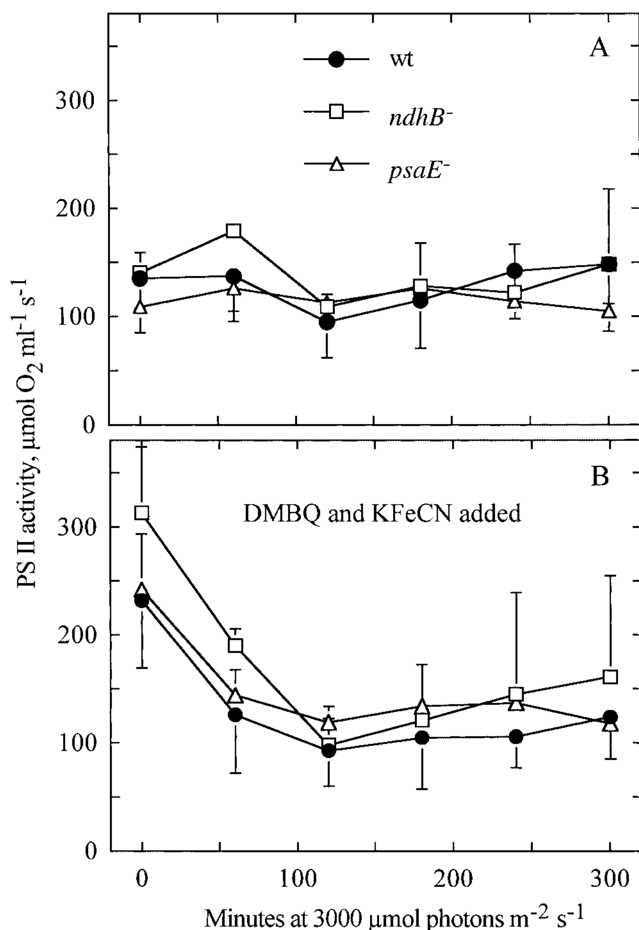

Fig. 1 Photoinhibition of PSII without spectinomycin. Points represent gross oxygen evolution by *Synechocystis* sp. PCC 6803 after various times of exposure to 3,000 µmol photons m^{-2} s^{-1} PAR. Net oxygen evolution was measured in saturating light (1,500 µmol photons m^{-2} s^{-1}) and corrected for dark respiration to yield gross oxygen evolution. Points are means of data from 5 to 7 photoinhibition experiments. Error bars represent standard deviations of the sample. Filled circles, wild type; open squares, *ndhB*$^-$ mutant; open triangles, *psaE*$^-$ mutant. A, no additions; B, 100 µM DMBQ and 100 µM ferricyanide added to uncouple PSII from downstream PET.

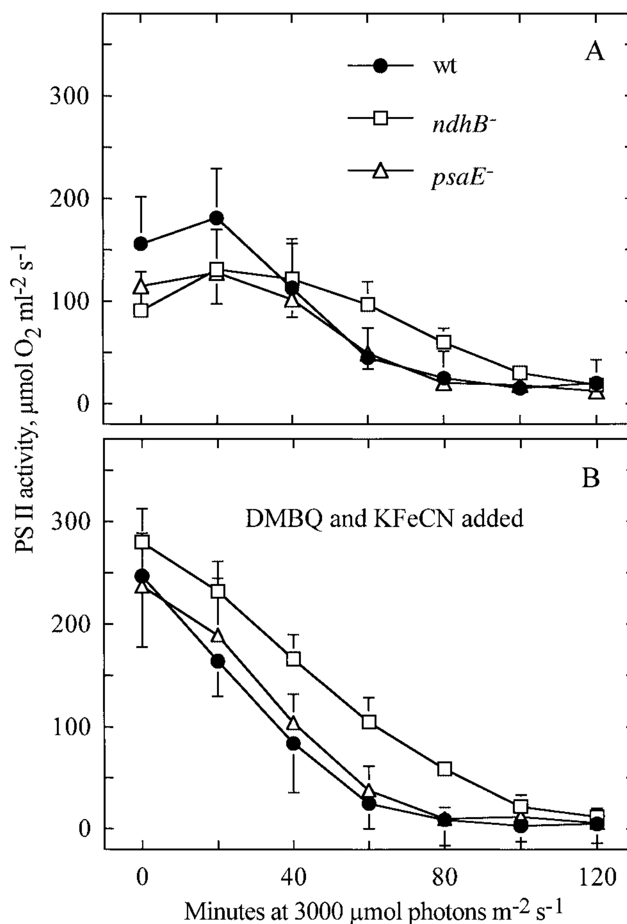

Fig. 2 Photoinhibition of PSII activity with addition of 75 µM spectinomycin. Points represent gross oxygen evolution by *Synechocystis* sp. PCC 6803 after various times of exposure to 3,000 µmol photons m^{-2} s^{-1} PAR in the presence of 75 µM spectinomycin. Net oxygen evolution was measured in saturating light (1,500 µmol photons m^{-2} s^{-1}) and corrected for dark respiration to yield gross oxygen evolution. Points are means of data from 5 to 7 photoinhibition experiments. Error bars represent standard deviations of the sample. Filled circles, wild type; open squares, *ndhB*$^-$ mutant; open triangles, *psaE*$^-$ mutant. A, no additions; B, 100 µM DMBQ and 100 µM ferricyanide added to uncouple PSII from downstream PET.

Fig. 5 show that the light-induced increase in reduction of $P700^+$ with addition of DCMU is strongly inhibited by 100 µM MV in both the mutant and the wild-type strains, confirming that it is an acceleration of PSI cyclic electron transport. Reduction of $P700^+$ that is not inhibited by DCMU and methyl viologen likely represents electron input to PSI from respiratory carbon metabolism via the NDH-1, succinate dehydrogenases, or other electron donors to the PQ pool (Cooley et al. 2000).

Discussion

Our experiments show that *Synechocystis* mutants defective in one of the multiple pathways of PSI cyclic electron transport are not sensitized to photoinhibition of PSII. Rates of PSII photoinhibition were very similar in mutant and wild-type strains, both in the presence and in the absence of the translation inhibitor spectinomycin (Fig. 1, 2). This result indicates that both damage to PSII and simultaneous repair by D1 synthesis proceeded at similar rates in the mutant and wild-type strains. We propose that in both *ndhB*$^-$ and *psaE*$^-$ mutants, the remaining PSI cycles are able to compensate for the absent PSI cycle and fulfil whatever role cyclic electron transport plays in limiting photoinhibition of PSII. Alternatively, the cyclic-deficient mutants may have functionally complemented themselves such that sensitivity to photoinhibition caused by lack of

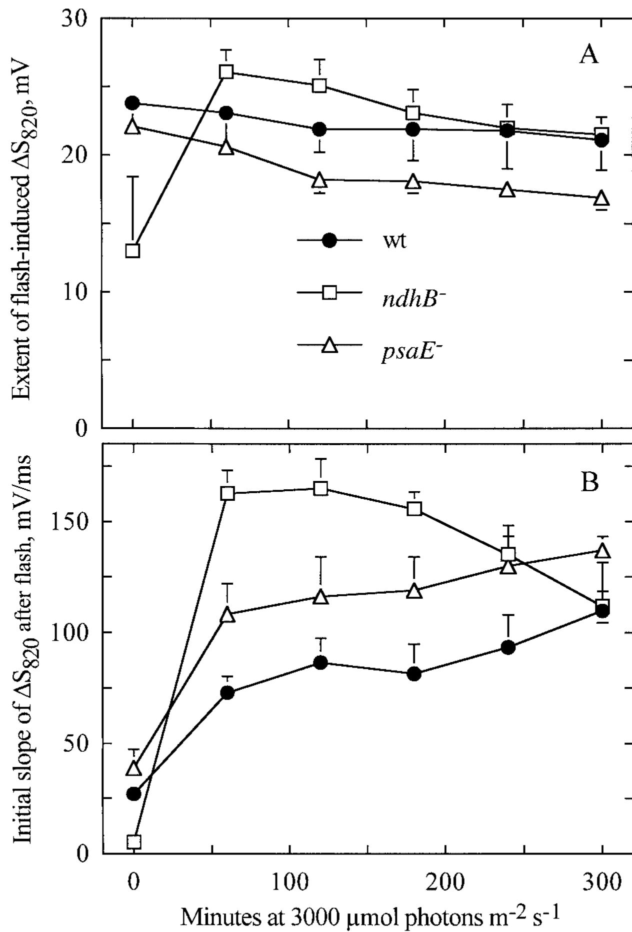

Fig. 3 Effect of strong light on PSI activity without spectinomycin. PSI activity was measured in *Synechocystis* sp. PCC 6803 after various times of exposure to 3,000 µmol photons m^{-2} s^{-1} PAR. The flash used to oxidize P700 was 3 s of 1,000 µmol photons m^{-2} s^{-1} white light. The dark interval between flashes was 27 s. 25 µM DCMU was added for the measurement. Points are means of data from 5 to 7 photoinhibition experiments. Error bars represent standard deviations of the sample. Filled circles, wild type; open squares, *ndhB*⁻ mutant; open triangles, *psaE*⁻ mutant. A, extent of the ΔS_{820}, representing the quantity of photooxidizable P700 in the sample; B, initial slope of the ΔS_{820} in darkness after the flash, representing the rate of P700⁺ reduction by cyclic electron transport paths.

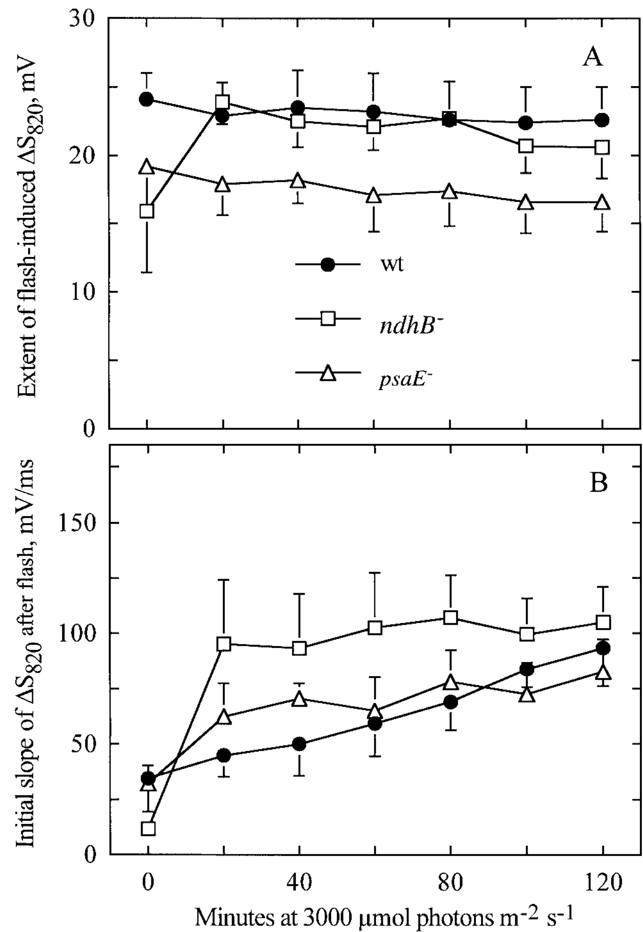

Fig. 4 Effect of strong light on PSI activity with 75 µM spectinomycin. PSI activity was measured in *Synechocystis* sp. PCC 6803 after various times of exposure to 3,000 µmol photons m^{-2} s^{-1} PAR in the presence of 75 µM spectinomycin. The flash used to oxidize P700 was 3 s of 1,000 µmol photons m^{-2} s^{-1} white light. Dark intervals between flashes were 27 s. 25 µM DCMU was added for the measurement. Points are means of data from 5 to 7 photoinhibition experiments. Error bars represent standard deviations of the sample. Filled circles, wild type; open squares, *ndhB*⁻ mutant; open triangles, *psaE*⁻ mutant. A, extent of the ΔS_{820}, representing the quantity of photooxidizable P700 in the sample; B, initial slope of the ΔS_{820} in darkness after the flash, representing the rate of P700⁺ reduction by cyclic electron transport paths.

a PSI cycle is offset by upregulation of other photoprotective mechanisms. For example, both the *ndhB*⁻ and *psaE*⁻ mutants show a slightly higher ratio of total carotenoids to chl *a* than the wild type strain when grown in moderate light intensity (Table 1).

Our results differ from studies of tobacco mutants lacking the chloroplast NdhB protein. Endo et al. (1999) found one such mutant to be sensitized to photoinhibition and attributed this phenotype to a low ATP:NADPH ratio that slowed the Calvin cycle. Linear electron transport alone is known to generate a more than adequate ATP:NADPH ratio for operation of the Calvin cycle (Furbank et al. 1990, Heber and Walker 1992), but there are many uses for ATP in the chloroplast. For example, Horváth et al. (2000) observed that another tobacco mutant lacking the chloroplast NdhB grew poorly under conditions that favored photorespiration, a process that requires ATP. Our results with cyanobacteria showed no sensitivity to photoinhibition in an *ndhB*⁻ mutant but they do not necessarily conflict with the tobacco studies. The *ndhB*⁻ mutant of *Synechocystis* sp. PCC 6803 showed a dramatic increase in PSI-driven cyclic electron transport induced by strong light. This effect was not reported for the tobacco mutants.

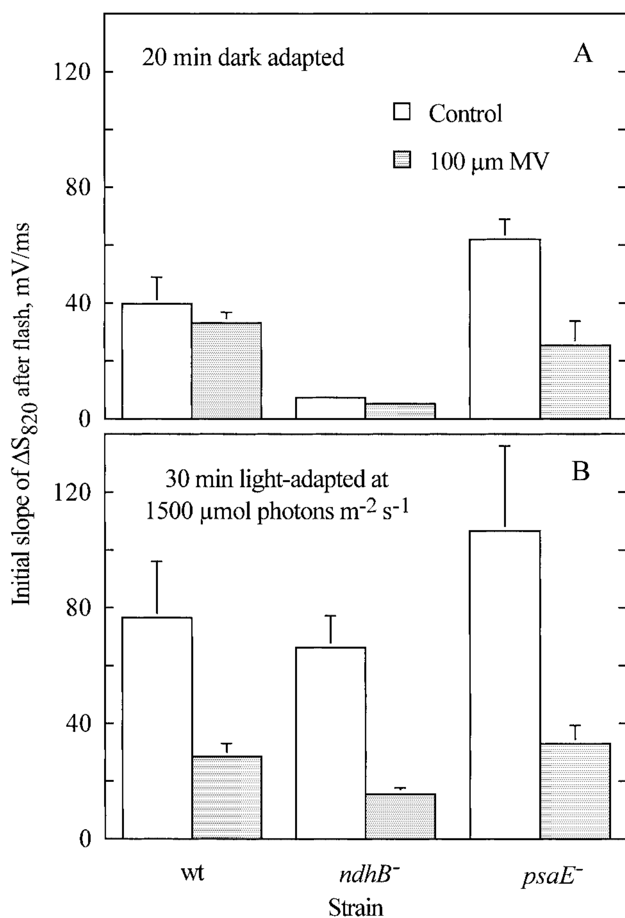

Fig. 5 The effect of 100 µM methyl viologen on the acceleration of PSI cycles by strong light. The initial rate of $P700^+$ reduction following an oxidizing flash was measured in *Synechocystis* sp. PCC 6803 before and after exposure to 1,500 µmol photons m^{-2} s^{-1} PAR for 30 min. 25 µM DCMU was added for all measurements. Values are mean results from five separate experiments. Error bars represent standard deviations of the sample. Open columns, no methyl viologen present during the measurement; filled columns, 100 µM methyl viologen present during the measurement. A, samples dark-adapted for 20 min; B, samples exposed to 1,500 µmol photons m^{-2} s^{-1} PAR for 30 min.

A significant result of the present study is the observation of a robust PSI cycle induced by strong light in the *ndhB*$^-$ mutant (Fig. 3B, 4B). Previous studies of the *ndhB*$^-$ mutant of *Synechocystis* sp. PCC 6803 found very low rates of PSI cyclic electron transport and concluded that all PSI-driven cyclic electron transport occurred via the NDH-1 complex in cyanobacteria (Mi et al. 1992). In contrast, study of *psaE*$^-$ and *ndhF*$^-$ mutants of *Synechococcus* sp. PCC 7002 indicated that both NDH-1 dependent and PsaE dependent PSI cycles occur in cyanobacteria (Yu et al. 1993). In tobacco, the biphasic inhibition of plastoquinone reduction by antimycin A was also interpreted as evidence for the existence of at least two PSI cycles (Endo et al. 1998). Most recently, study of a *Synechocystis* 6803 mutant in which the membrane-association of ferredoxin-NADP oxidoreductase was abolished in an *ndhB*$^-$ strain also supported the existence of a PSI cycle that was independent of NDH-1 (van Thor et al. 2000). Our observation of a light-induced PSI cycle in the *ndhB*$^-$ mutant confirms the occurrence of at least two PSI-driven cycles in cyanobacteria, one of which does not require the NDH-1.

Acceleration of PSI cyclic electron transport has been observed previously in cyanobacteria exposed to strong light or to salt stress (Jeanjean et al. 1993, Herbert et al. 1995, van Thor et al. 2000). In the case of strong light, cultures of *Synechococcus* sp. PCC 7942 grown in 60 and 350 µmol photons m^{-2} s^{-1} showed much faster rates of PSI cyclic electron transport than cultures grown in 17 µmol photons m^{-2} s^{-1}. Addition of 100 µM MV, which competitively inhibits the reduction of ferredoxin by PSI, inhibited the slow PSI cycles of the weak light cultures by half (Herbert et al. 1995). In the present study, weak light cultures exposed suddenly to strong light showed an acceleration of PSI cyclic electron transport that was sensitive to 100 µM MV (Fig. 5). This result confirms that ferredoxin is an electron carrier in the accelerated cycle and that the acceleration was not caused by increased donation of electrons to the PQ pool from respiratory carbon metabolism. In addition to this conclusion, comparison of Fig. 3B and 4B shows that the translation inhibitor spectinomycin partly inhibited acceleration of the PSI cycles by strong light, suggesting that new protein synthesis is required for complete acceleration. Alternatively, inhibition of protein synthesis by spectinomycin may have generally reduced consumption of ATP by the cells and caused a feedback inhibition of cyclic electron transport by lack of ADP.

PsaE is thought to be required for the FQR pathway of PSI cyclic electron transport (Yu et al. 1993). PsaE is known to aid in the binding of ferredoxin to the reducing side of PSI (Rousseau et al. 1993, Sonoike et al. 1993) and lack of PsaE has been shown to greatly reduce the lifetime of the PSI/ferredoxin complex (Barth et al. 1998). Despite this, *psaE*$^-$ mutants show normal rates of linear PET and NDH-1 dependent cyclic electron transport, both of which require ferredoxin (Chitnis et al. 1989, Zhao et al. 1993, Yu et al. 1993, Xu et al. 1994). This apparent contradiction can be reconciled if prolonged association of ferredoxin with PSI is required for the FQR path of cyclic electron transport but not for reduction of $NADP^+$. In this case, loss of PsaE could inhibit the FQR cycle by reducing the lifetime of the PSI/ferredoxin complex without inhibiting either the NDH-1 cycle or linear electron transport. Our study shows that the PSI cyclic electron transport of the *psaE*$^-$ mutant of *Synechocystis* sp. PCC 6803 often exceeded that of the wild type (Fig. 3B, 4B). A photoacoustic study of the *psaE*$^-$ mutant of *Synechococcus* sp. PCC 7002 also found PSI-cyclic electron transport rates that were equal to the wild-type (Charlebois and Mauzerall 1999). These findings indicate an increased NDH-1 cycle in *psaE*$^-$ mutants that may compensate for lack of the

FQR cycle.

The activity of PSI is not inhibited by strong light even when new protein synthesis is inhibited (Fig. 3, 4). This result confirms earlier reports that PSI is much less sensitive to strong light than is PSII (Powles 1984, Kyle et al. 1984, Canaani et al. 1989, Havaux and Eyeletters 1991, Martin et al. 1997). The insensitivity of PSI to strong light is interesting in the case of the *psaE*$^-$ mutant because loss of PsaE renders PSI unstable at elevated temperature (Golbeck and Bryant 1991, Charlebois and Mauzerall 1999). Our results show that the thermal sensitivity of PSI caused by loss of PsaE is not accompanied by sensitivity to strong light.

The occurrence of photoprotective energy dissipation in the *ndhB*$^-$ and *psaE*$^-$ mutants of *Synechocystis* sp. PCC 6803 was not tested in our study. With respect to the phenomenon of state transitions, the *ndhB*$^-$ mutant has been observed to be "locked" in State 1 (Schreiber et al. 1995), meaning that transfer of excitation from PSII to PSI cannot be increased under lighting conditions that overexcite PSII. The photoprotective value of the State 1 to State 2 transition in cyanobacteria has never been convincingly demonstrated but is plausible. Our observations suggest that a State 1 to State 2 transition may occur in the *ndhB*$^-$ mutant when PSI cyclic electron transport is induced by strong light. Future study will address this issue. Construction of an *ndhB*$^-$/*psaE*$^-$ double mutant in *Synechocystis* sp. PCC 6803 will also be helpful to understanding the roles of the PSI cyclic electron transport pathways in tolerance of excessive light and other stresses.

Materials and Methods

Culture and experimental conditions

Cultures of wild-type, *ndhB*$^-$ and *psaE*$^-$ strains of *Synechocystis* sp. PCC 6803 were grown in 50 ml tubes of BG-11 liquid culture medium (Sigma, St. Louis, Missouri, U.S.A.) supplemented with 10 mM sodium bicarbonate and having a pH of approximately 8. Cultures were incubated at 27°C, sparged with 3% CO_2 in air, and illuminated with cool white fluorescent light at 15–35 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR). Light intensity was measured using an LI-189 quantum sensor (Li-Cor, Lincoln, Nebraska, U.S.A.). The *ndhB*$^-$ mutant of *Synechocystis* sp. PCC 6803 (mutant M55, Ogawa 1991, Mi et al. 1992) was provided by Professor T. Ogawa of Nagoya University. A *psaE*$^-$ mutant of *Synechocystis* sp. PCC 6803 (Chitnis et al. 1989) was provided by Professor Parag Chitnis of Iowa State University. An additional *psaE*$^-$ mutant of *Synechocystis* sp. PCC 6803 was provided by Professor Wim Vermaas of Arizona State University. In this latter mutant, a region from 61 bp upstream to 92 bp downstream of the 5′ end of *psaE* was replaced with a restriction fragment conferring resistance to chloramphenicol. The two *psaE*$^-$ strains were phenotypically identical in our photoinhibition experiments. The data presented in this paper are from the *psaE*$^-$ mutant provided by Professor Vermaas.

Growth measurements

Light scattering was used to monitor culture density and was measured as apparent absorbance at 750 nm in a Spectronic 20 spectrophotometer (Spectronic Instruments, Milford, Pennsylvania, U.S.A.). For growth measurements, rapidly growing cultures were centrifuged, resuspended in fresh BG-11 to A_{750} = 0.1, and illuminated at 30 µmol photons m^{-2} s^{-1} PAR. Aliquots of 3 ml were removed at 24 to 48 h intervals and A_{750} was measured to determine culture density.

Pigment measurements

Photosynthetic pigments were measured using an Aminco DW-2000 scanning spectrophotometer (SLM/Aminco, Urbana, Illinois, U.S.A.). Cultures were diluted to A_{750} = 0.5 and grown for 48 h under the conditions described above. Samples from these cultures were then diluted again to A_{750} = 0.5 before pigment measurements were made. Phycocyanin concentrations were estimated by measuring A_{625} and A_{678} in whole cell suspensions and applying the calculations of Myers et al. (1980). Chl *a* and carotenoids were extracted in 100% acetone. Chl *a* in the acetone extract was quantified from absorbance at 663 nm using an absorption coefficient of 88.2 (mg Chl) *a* ml^{-1} cm^{-1}. The ratio of total carotenoids to Chl *a* in the acetone extract was estimated by comparing the integrated absorbance of the 400–520 nm and 640–690 nm regions of the absorption spectrum. Purified Chl *a* from *Anacystis nidulans* (Sigma) was used as a reference for this ratio.

Photoinhibition

Light stress treatments were given in 100 ml water-jacketed beakers maintained at 27°C and sparged with 3% CO_2 in air. Cultures were diluted to A_{750} = 0.5, added to the water-jacketed beakers, and dark-adapted for 20 min before the photoinhibitory treatment commenced. The beakers were stirred continuously during treatments. Tungsten slide projector lamps (CAL-type, General Electric, Cleveland, Ohio, U.S.A.) provided photoinhibitory light of 3,000 µmol photons m^{-2} s^{-1} PAR. Activities of PSII and PSI were measured concurrently in aliquots removed from the beakers at intervals. To block photoinhibition repair by D1 protein turnover, 75 µM spectinomycin was used to inhibit translation. The *ndhB*$^-$ and *psaE*$^-$ mutants used in this study possess kanamycin and chloroamphenicol resistance markers, respectively, but both are sensitive to spectinomycin.

PSII activity

Oxygen evolution by PSII was monitored in intact cells with a Hansatech DW1 liquid-phase O_2 electrode and water-jacketed cuvette maintained at 27°C. Actinic light for O_2 measurements was 1,500 µmol photons m^{-2} s^{-1} PAR from an EKE halogen projector lamp. One ml aliquots from the photoinhibition beakers were placed in the O_2 electrode cuvette and dark-adapted for 5 min. The rate of O_2 consumption in darkness was then recorded for 2 to 5 min to measure respiration. Subsequently, actinic light was turned on and O_2 production was measured for 3 min. Following this, DMBQ and KFeCN were added to a final concentration of 100 µM each to uncouple PSII activity from the rest of electron transport (Mulo et al. 1998). Uncoupled O_2 evolution was then measured for 2 min. O_2 evolution rates were corrected for dark respiration to yield gross oxygen evolution by PSII. Dark respiration rates were consistently less than 10% of gross oxygen evolution rates.

PSI activity

The photooxidation and dark reduction kinetics of P700 were measured in intact cells using the absorbance change at 820 nm as described elsewhere (Herbert et al. 1995, Thomas et al. 1998, Thomas et al. 1999). This absorbance change was monitored by reflectance using a Walz modulated detection system (Effeltrich, Germany) consisting of a PAM 101 control unit and an ED 800T emitter-detector unit. The light-induced changes in the 820 nm reflectance signal (ΔS_{820}) were taken as proportional to changes in the P700 oxidation state. White actinic flashes (1,000 µmol photons m^{-2} s^{-1}) were 3 s in length and separated by 27 s of darkness. Four or more flash-induced

transients were signal-averaged for each measurement. Samples for ΔS_{820} measurements were prepared by filtering 10 ml of culture onto 0.45 μm membrane filters (Millipore type HA). The filter and cells were then placed under an acrylic light guide connected to the Walz system by a branched fiber optic cable. Inhibitors (DCMU, methyl viologen) were added to the samples prior to filtration. Since cultures were diluted to an equal cell density prior to filtration, ΔS_{820} parameters were taken to indicate PSI abundance and activity relative to cell number. The change in 820 nm reflectance signal caused by the actinic light was about 12% of the total 820 nm reflectance signal of the samples.

Reflectance signal transients at 820 nm were analyzed and interpreted as described previously (Thomas et al. 1998, Thomas et al. 1999). The light-saturated extent of the ΔS_{820} was taken to indicate the quantity of photooxidizable P700 in the sample. The initial slope of the ΔS_{820} immediately following the oxidizing flash was taken to indicate the rate of $P700^+$ reduction by upstream electron donors. Inhibitors (DCMU and methyl viologen) were used to block different inputs of electrons to $P700^+$, as has been done previously (Maxwell and Biggins 1976, Herbert et al. 1992, Herbert et al. 1995, Yu et al. 1993, Thomas et al. 1998, Thomas et al. 1999). Input from PSII was abolished with 25 μM DCMU. Methyl viologen at 100 μM was used to competitively inhibit reduction of ferredoxin by PSI and thus the ferredoxin-dependent PSI cycles. All measurements of P700 were made at room temperature.

Analysis

Quattro Pro (Corel Corporation, Ottawa, Ontario, Canada) was used for data reduction and numerical analyses. Differences between samples were tested for significance with the Student's *t*-test using an α of 5%. All experiments were replicated at least five times using different starting cultures.

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