

A Comprehensive Analysis of the Peroxiredoxin Reduction System in the Cyanobacterium *Synechocystis* sp. Strain PCC 6803 Reveals that All Five Peroxiredoxins Are Thioredoxin Dependent^{∇†}

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Cyanobacteria perform oxygenic photosynthesis, which gives rise to the continuous production of reactive oxygen species, such as superoxide anion radicals and hydrogen peroxide, particularly under unfavorable growth conditions. Peroxiredoxins, which are present in both chloroplasts and cyanobacteria, constitute a class of thiol-dependent peroxidases capable of reducing hydrogen peroxide as well as alkyl hydroperoxides. Chloroplast peroxiredoxins have been studied extensively and have been found to use a variety of endogenous electron donors, such as thioredoxins, glutaredoxins, or cyclophilin, to sustain their activities. To date, however, the endogenous reduction systems for cyanobacterial peroxiredoxins have not been systematically studied. We have expressed and purified all five *Synechocystis* sp. strain PCC 6803 peroxiredoxins, which belong to the classes 1-Cys Prx, 2-Cys Prx, type II Prx (PrxII), and Prx Q, and we have examined their capacities to interact with and receive electrons from the *m*-, *x*-, and *y*-type thioredoxins from the same organism, which are called TrxA, TrxB, and TrxQ, respectively. Assays for peroxidase activity demonstrated that all five enzymes could use thioredoxins as electron donors, whereas glutathione and *Synechocystis* sp. strain PCC 6803 glutaredoxins were inefficient. The highest catalytic efficiency was obtained for the couple consisting of PrxII and TrxQ thioredoxin. Studies of transcript levels for the peroxiredoxins and thioredoxins under different stress conditions highlighted the similarity between the PrxII and TrxQ thioredoxin expression patterns.

In oxygenic photosynthetic organisms, the high oxidizing potential created in photosystem II during light-driven water splitting and the concomitant oxygen evolution, which takes place in the vicinity of electron transport reactions, exacerbates the problem of reactive oxygen species (ROS) production posed by normal aerobic metabolism (3, 37). Unleashed, these reactive compounds may oxidize and irreversibly inactivate components of the photosynthetic apparatus. Such inactivation, referred to as photoinhibition, is particularly apparent under conditions of high light intensity, nutrient deprivation, or cold, when the rate of ROS production is high due to the absorption of excess light energy (66). Therefore, chloroplasts and cyanobacteria, which are phylogenetically related, must be equipped with antioxidant enzymes, such as superoxide dismutases and peroxidases, that restrict the levels of ROS produced during photosynthesis. Despite their common origin and the similar nature of the processes leading to the formation of ROS in cyanobacteria and chloroplasts, current knowledge implies that the strategies they use to control the levels of, e.g., peroxides may be quite different (5).

In chloroplasts, ascorbate peroxidases are principal scavengers

of H₂O₂, and ascorbate concentrations are as high as 12 to 25 mM (15). In contrast, ascorbate concentrations in cyanobacteria are much lower, ranging from 20 to 100 μM (67), and genes encoding ascorbate peroxidases are missing from the sequenced cyanobacterial genomes, including that of *Synechocystis* sp. strain PCC 6803. Glutathione concentrations in cyanobacteria range from 2 to 4 mM, but no glutathione-dependent peroxidase activity has been detected in any species tested from this phylum (67, 68). Catalases of plants and algae are located mainly in the peroxisomes but not in plastids. However, catalase activities have been found in all cyanobacterial species examined (38, 39, 43). A *Synechocystis* mutant devoid of its only catalase-peroxidase (*katG*) provided a useful tool for studying the nature of the remaining peroxidase activities (69). This mutant revealed the significance of a light-dependent thiol-specific peroxidase activity, raising the possibility that peroxiredoxins (Prx), which could benefit from thioredoxins (Trx) as electron donors, might be responsible for part of the peroxide decomposition in this organism (69).

Trx constitute a family of redox-active enzymes, which in cyanobacteria and chloroplasts may receive reducing equivalents directly from the linear photosynthetic electron flow by means of ferredoxin and ferredoxin Trx reductase (7, 14, 35, 59). Subsequently, Trx catalyze the reduction of a variety of target enzymes, including Prx, by disulfide/dithiol exchange (7). Prokaryotic phylogenetic groups of Trx include the *m*, *x*, and *y* types, all of which have homologues in the chloroplast of plants and algae (35). The completely sequenced genome of *Synechocystis* encodes one Trx from each of these families. TrxA (open reading frame [ORF] slr0623) is of the *m* type (40); TrxB (slr1139) is of the *x* type; and TrxQ (slr0233) is of the *y* type

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(14, 48). Our previous studies of *Synechocystis* deletion mutants lacking either TrxB or TrxQ have shown that the absence of TrxQ, but not that of TrxB, confers hypersensitivity to hydrogen peroxide, suggesting a role for TrxQ in peroxide tolerance in this organism (47). TrxA has been found to be essential in *Synechocystis*, and no deletion mutants could be obtained (42).

Prx comprise a class of peroxidases that have been extensively studied in *Saccharomyces cerevisiae*, mammals, and non-photosynthetic bacteria (53, 72), as well as in plants (11, 12, 58). Based on their phylogeny and catalytic mechanisms, Prx are classified into four groups: 1-Cys Prx, 2-Cys Prx, type II Prx (PrxII), and Prx Q (11, 58, 72). The genome of the cyanobacterium *Synechocystis* encodes five Prx (64), which belong to each of the established classes 2-Cys Prx (sl10755), 1-Cys Prx (slr1198), PrxII (sl11621), and Prx Q (slr0242, sl10221). Studies of cyanobacterial Prx mutant strains suggest that these enzymes function in adaptation to growth at elevated light intensities, though the mechanism may not always involve simply peroxide detoxification. A cyanobacterial 2-Cys Prx homologue, highly similar to chloroplast BAS1 (4), was found to be required for optimal growth under high light conditions in *Synechocystis* (26) as well as in a *Synechococcus* sp. strain PCC 7942 (46), although it did not affect the ability to survive high concentrations of H₂O₂ added to cell cultures (46). The *Synechocystis* KatG 2-Cys Prx double mutant was impaired in translation of the photosystem II D1 protein during the repair process following high-light-induced damage (42). A *Synechocystis* PrxII disruptant strain showed a severely reduced growth rate relative to that of the wild type strain under normal light (23, 27), whereas a *Synechocystis* 1-Cys Prx disruption mutant grew somewhat more slowly than the wild-type strain but was not particularly sensitive to H₂O₂ or methyl viologen (23). In contrast, an *Anabaena* sp. strain PCC 7120 mutant failing to express one of its four Prx Q was hypersensitive to methyl viologen and grew poorly at moderate light intensities (29).

Numerous studies have explored the sources of reducing equivalents for Prx. For example, the 2-Cys Prx AhpC from some nonphotosynthetic bacteria is reduced by the flavoprotein AhpF (51), an NADPH disulfide oxidoreductase, which is absent from cyanobacteria and plants. The plant chloroplast 2-Cys Prx has instead been found to receive electrons from CDSP32, a protein containing two Trx domains in tandem (6), and NTRC, which consists of an NADPH Trx reductase (NTR) and a Trx in a single polypeptide chain (49). However, no homologue of CDSP32 can be found in cyanobacteria, and only some cyanobacterial species, such as *Anabaena* sp. strain PCC 7120, but not *Synechocystis*, harbor homologues of NTRC (14). Plastid 2-Cys Prx and Prx Q have also been reported to receive reducing equivalents from presumably ferredoxin Trx reductase-dependent, simple-module Trx of prokaryotic origin, such as Trx *x* (9) and Trx *y* (10). Even a cyclophilin (13) and glutaredoxin (Grx) (57) have been suggested as potential plant Prx electron donors. The 10 Prx of the plant *Arabidopsis thaliana* and its many Trx and Trx-like proteins are distributed among different cellular compartments (11, 12), whereas the five *Synechocystis* Prx, with the possible exception of PrxQ2, might be found in the same compartment as the Trx. Therefore, a priori, until further localization has been demonstrated, any of the *Synechocystis* Trx, or perhaps Grx or glutathione, as

suggested in reference 23, could act as electron donors for these Prx. The *Synechocystis* *x*-type Trx has been reported to be a poor electron donor for *Synechocystis* 1-Cys Prx and PrxII (23). *Synechocystis* 2-Cys Prx (73) and five of the *Synechococcus elongatus* PCC 7942 Prx (63) have been shown to catalyze the decomposition of peroxides in vitro only by using NTR and Trx from *Escherichia coli* as sources of reducing equivalents. Until now, no systematic study of the endogenous reduction systems for cyanobacterial Prx has been performed, and none of the existent reports on cyanobacterial Prx activity compare the whole set of Prx in one cyanobacterium.

To better understand the mechanisms for peroxide decomposition in cyanobacteria, we aimed at clarifying the nature of the cyanobacterial Prx reduction systems. Therefore, we have examined the interactions of all five *Synechocystis* Prx with the three different Trx from this organism with regard to affinity and catalytic efficiency. Furthermore, a role for the *Synechocystis* Grx and glutathione as electron donors for these Prx was considered. Finally, we analyzed the expression of the *prx* and *trx* genes under various conditions (e.g., after a shift to high light intensity, addition of hydrogen peroxide, moderate heat shock, or nitrogen deprivation) in order to search for possible correlations between their distinct expression patterns.

MATERIALS AND METHODS

Growth of *Synechocystis* sp. strain PCC 6803 cultures. Cells were grown photoautotrophically at 30°C in BG11 medium (54) supplemented with 1 g/liter NaHCO₃ and were bubbled with a stream of 1% (vol/vol) CO₂ in air under continuous illumination at a light intensity of 50 to 70 μE · m⁻² · s⁻¹. All treatments were performed using exponentially growing *Synechocystis* sp. strain PCC 6803 cultures, typically displaying a chlorophyll concentration of 3 to 4 μg/ml. For high light conditions, cultures were illuminated with white light at an intensity of 500 μE · m⁻² · s⁻¹, and the temperature was kept at 30°C by applying a 5-cm-thick water filter. For heat shock conditions, cells were grown in a water bath at 43°C. For oxidative stress conditions, 1 mM hydrogen peroxide was added. For nitrate starvation, cells were harvested by centrifugation at 9,000 × *g* for 10 min, washed three times with BG11 medium without nitrate, and resuspended in the same medium.

Sequences of genes and proteins. Gene and protein sequences were obtained from the CyanoBase (<http://genome.kazusa.or.jp/cyanobase>). The *trx* genes were named *trxA* (ORF slr0623), *trxB* (slr1139), and *trxC* (slr0233). The *prx* genes were 2-cys *prx* (sl10755), *prxII* (sl11621), 1-cys *prx* (slr1198), *prxQ2* (sl10221), and *prxQ1* (slr0242).

RNA isolation and Northern blot analysis. Total RNA was isolated from 40-ml samples of *Synechocystis* sp. strain PCC 6803 cultures subjected to the treatments described above and at the times indicated. Cells were harvested by centrifugation and were broken using acid-washed baked glass beads (Sigma-Aldrich) in the presence of phenol and chloroform as previously described (19). For Northern blot analysis of mRNA levels, 10 μg of total RNA from each sample was electrophoresed on denaturing 1.5% agarose gels. Transfer of the RNA to nylon membranes (Millipore), prehybridization, hybridization, and washes were performed as recommended by the manufacturer. Probes for all genes were obtained by PCR, where 5 μg DNA of each *trx*, *prx*, and control gene was labeled with ³²P using a Ready-To-Go kit (GE Healthcare) according to the manufacturer's instructions. As a control, filters were reprobated with a 580-bp HindIII and BamHI fragment from plasmid pAV1100 containing the constitutively expressed RNase P RNA gene (*nmpB*) from *Synechocystis* sp. strain PCC 6803 (70). The *isiA*, *hspA*, and *pgr5* genes, used as indicators of the different stress conditions, were amplified using the primers listed in Table 1. The *glnN* gene was used as a control for nitrogen deprivation (52). Relative transcript levels were determined with a Cyclone Plus Storage Phosphor system (Perkin-Elmer).

Cloning, expression, and purification of recombinant proteins. DNA fragments corresponding to the entire coding region of each *prx* gene (sl10755, sl11621, slr1198, sl10221, and slr0242) were amplified by PCR using appropriate forward and reverse primers, described in Table 1, and the High Expand Fidelity kit (Roche). All PCR products were cloned into the pET28a vector (Novagen), except slr1198, which was cloned into the pET24a vector, in order to produce

TABLE 1. Oligonucleotides used for amplifying thioredoxin, peroxiredoxin, glutaredoxin, and control genes used as probes

Oligonucleotide	Sequence (5'-3')
TrxAFTTCCAGTATGAGTGCTACCC
TrxARAAGCCAGCGCTTAAAGATAT
TrxBFGACCATTATGAGTTTAC
TrxBRCAAATAGCTCGAGTCAGTTTAGGG
TrxQFACTGGATTAATATCTTTGCG
TrxQRCTAAAGGTTTGTGTGTCG
2CysFGCTACATATGACAGAGGTATTAAGGGTAG
2CysRGCTACTCGAGCTAAGGTTCCGCCACTGTCTC
PrxIIFGAGGTACATATGACCCCGAACG
PrxIIRGGTTTCACTCGAGTAATCAC
1CysFGCTACATATGGCCTTACAACCTCGTGATG
1CysRGCTACTCGAGCTTATTGGGTTGGGGGGTCT
PrxQ2FCCTGGGGCATATGCAACCAGAGTTG
PrxQ2RCGGGATTATTTACGTCGACGGGGAG
PrxQ1FGAATTTTTTTCATATGGCCACTGCCTTAG
PrxQ1RCCAAGGCTCGAGGATTTGCTTGC
GrxAFGGCGTAGAGATATGACCGGTC
GrxARGTTGGATCCGCTGTCTCGGCCAAAAATTG
GrxBFCTGGGAATTCACGGGCAC
GrxBRGGTGATCATATGGCTAATTTGTTC
Pgr5FGGAGTCACTCATATGTTTCGCC
Pgr5RCTCAGTTTCTCGAGAATTATTG
HspAFCCACACATCAGGAGTTAATCAT
HspARTTGATCATCTGAGGTCAGGAGC
IsiAFCATAGGTCTCGGGTGGAC
IsiARTAAAGCTGATGGCTAATG

recombinant proteins with N-terminal histidine tags. The resulting plasmids were named p2Cys, pPrxII, p1Cys, pPrxQ2, and pPrxQ1 and encoded the 2-cys prx, prxII, 1-cys prx, prxQ2, and prxQ1 genes, respectively. For expression of Prx, *Escherichia coli* (strain BL21) cells were transformed and grown in LB medium supplemented with kanamycin. Expression was induced by adding 1 mM isopropyl- β -D-thiogalactose (IPTG) at an optical density at 600 nm of about 0.4. The recombinant proteins were purified by nickel affinity chromatography using a HiTrap chelating HP column (GE Healthcare), and thereafter, gel filtration was performed on a Superdex 75 Hiload 16/60 column (GE Healthcare).

The wild-type version of TrxA was purified as described previously (41). For wild type TrxB and TrxQ, DNA fragments corresponding to the coding regions of *trxB* (slr1139) and *trxQ* (slr0233) were amplified by PCR using primers TrxBF and TrxBR or primers TrxQF and TrxQR, respectively (Table 1). PCR products were cloned into the pET28a vector, and recombinant TrxB and TrxQ were purified by nickel affinity chromatography and gel filtration as described for the Prx. His-tagged cysteine-to-serine mutants of TrxA, TrxB, and TrxQ, designated TrxA(C35S), TrxB(C34S), and TrxQ(C33S), respectively, were purified as described previously (32, 48).

The *grxA* (ssr2061) and *grxB* (slr1562) genes were amplified by PCR using primer pairs GrxA4-GrxA5 and GrxB-F2-GrxB-R2, respectively (Table 1). Fragments were cloned into pET28a, and the expressed GrxA and GrxB proteins were purified as described for the Prx.

Protein concentrations were determined according to reference 33.

SDS-PAGE and Western blot analysis. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10 or 12% acrylamide concentration and were stained with Coomassie brilliant blue (Sigma-Aldrich). For solubilization under reducing conditions, the loading buffer included 100 mM dithiothreitol (DTT) and 5% (vol/vol) β -mercaptoethanol. For nonreducing conditions, DTT and β -mercaptoethanol were omitted from the loading buffer, and 50 mM iodoacetamide was included. All samples were boiled for 5 min prior to electrophoresis. For Western blot analysis, proteins were resolved on SDS-PAGE (15% acrylamide) gels, transferred to nitrocellulose membranes (Bio-Rad), and probed with anti-TrxA (1:1,000), anti-TrxB (1:10,000), or anti-TrxQ (1:5,000) antibodies as described previously (14). Signals were detected using an anti-rabbit secondary antibody (Sigma-Aldrich) and the ECL-Plus immunoblotting detection system (GE Healthcare).

Peroxidase assay. Peroxide decomposition catalyzed by Prx was measured using the ferrous ion oxidation (FOX) assay in the presence of xylenol orange as described in reference 71. The reduction of peroxides, such as hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (*t*-BOOH), or cumene hydroperoxide (CHP),

was measured kinetically with several time points during the initial linear rate of catalysis. The assay mixture typically contained 50 mM HEPES-NaOH (pH 7.0), 0.1 or 0.2 μ g/ μ l Prx, DTT at 0.2, 2, or 5 mM as indicated, and either 100 μ M H_2O_2 , 100 μ M *t*-BOOH, or 50 μ M CHP. Trx or Grx was included as an electron donor at the concentrations specified for each experiment and was kept reduced by addition of 0.2 mM DTT (Sigma Aldrich). To measure glutathione-dependent peroxidase activity, a reaction mixture containing 100 μ M reduced glutathione (GSH) (Sigma-Aldrich) was used. When the GSH-Grx system was tested, 100 μ M GSH, 0.15 U glutathione reductase from yeast (Sigma-Aldrich), 10 mM NADPH, and 4 μ M GrxA or GrxB were added to the reaction mixtures.

Peroxidase activity in the presence of 100 μ M GSH, 0.15 U glutathione reductase, and 10 mM NADPH was also measured indirectly by monitoring the rate of NADPH oxidation as described previously (23).

One unit of enzyme activity was defined as the amount of protein required to catalyze the reduction of 1 μ mol of H_2O_2 , *t*-BOOH, or CHP per min.

RESULTS

***Synechocystis* Prx-Trx protein-protein interactions.** In order to assess the interactions between the Prx and Trx in *Synechocystis* sp. strain PCC 6803, the His-tagged versions of 2-Cys Prx (sl10755), 1-Cys Prx (slr1198), PrxII (sl11621), and Prx Q (slr0242 and sl10221) were expressed in *Escherichia coli* and purified to homogeneity (Fig. 1A). The PrxQ protein encoded by ORF sl10221, here designated PrxQ2, was expressed without the 34-amino-acid N-terminal extension, which is predicted to be a signal peptide according to the SignalP, version 3.0 (<http://www.cbs.dtu.dk/services/SignalP/>), and TargetP, version 1.1 (<http://www.cbs.dtu.dk/services/TargetP/>), programs. Electrophoresis under denaturing conditions in the absence of a reductant showed that 2-Cys Prx and 1-Cys Prx migrate mainly as dimers; PrxQ2 and PrxQ1 migrate for the most part as monomers; and PrxII is distributed between dimeric and monomeric forms, though minor amounts of a few high-molecular-mass adducts could also be observed (Fig. 1B). Notably, the monomeric forms of PrxII, PrxQ2, and PrxQ1 migrated more rapidly under nonreducing than under reducing conditions (Fig. 1A and B). This would be consistent with the presence of intramolecular disulfides, which render the proteins more compact (74).

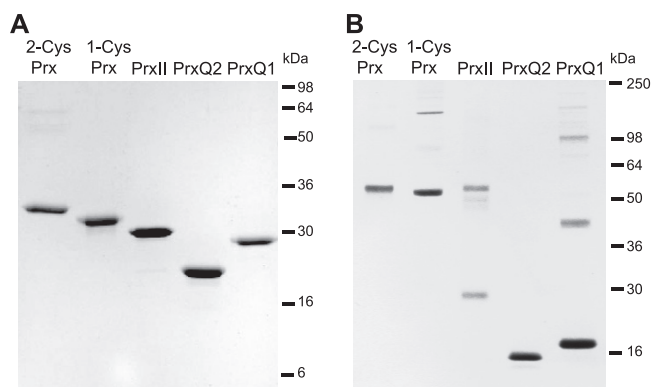


FIG. 1. *Synechocystis* sp. strain PCC 6803 peroxiredoxins. Four micrograms of each purified recombinant peroxiredoxin was incubated in the presence (A) or in the absence (B) of 100 mM DTT for 1 h on ice. Nonreduced samples (B) were solubilized in a buffer that included 50 mM iodoacetamide, whereas reduced samples (A) were solubilized in normal sample buffer. Thereafter, proteins were separated on 12% (A) or 10% (B) SDS-PAGE gels and stained with Coomassie brilliant blue.

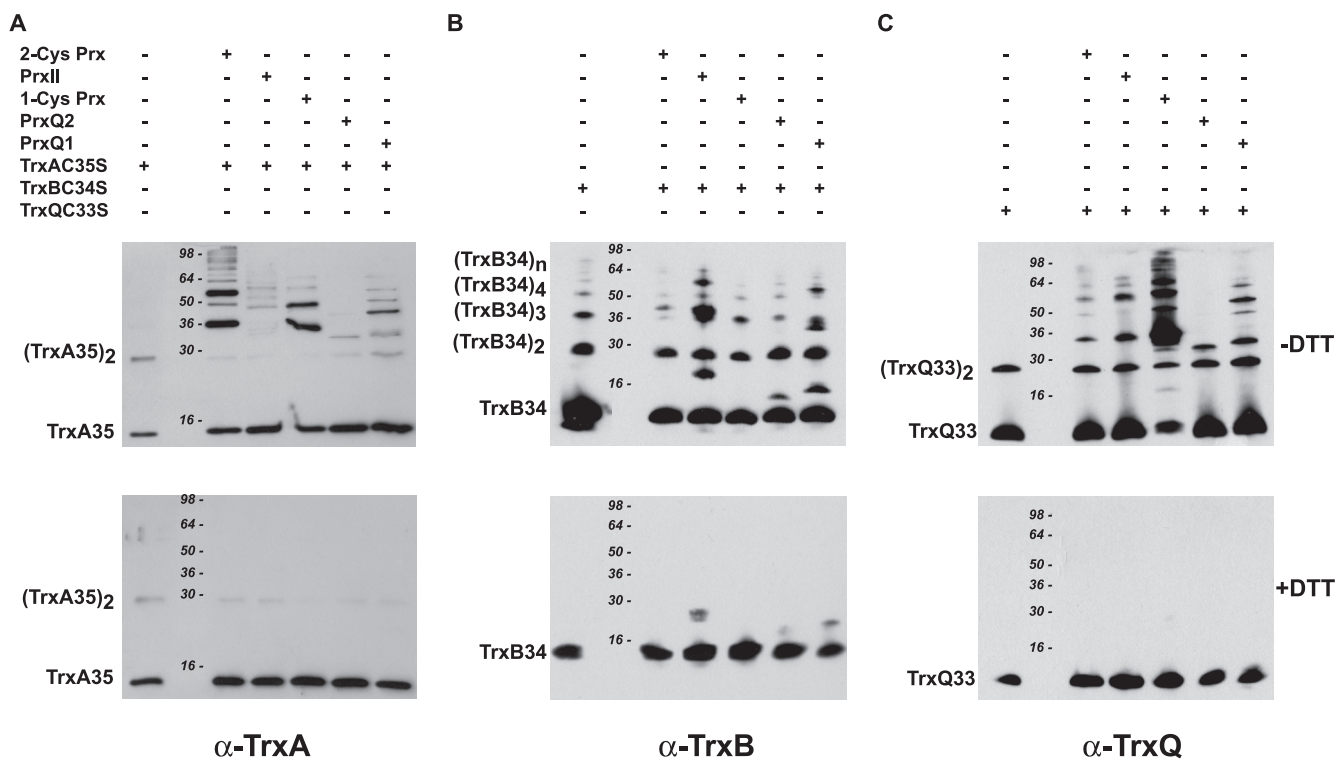


FIG. 2. Analysis of the thioredoxin-peroxiredoxin interaction. Twenty (A) or 11.25 (B and C) μg of each peroxiredoxin was incubated with 8 μg TrxA(C35S) (A), 4 μg TrxB(C34S) (B), or 4 μg TrxQ(C33S) (C). Thereafter, proteins were resolved on 12% SDS-PAGE gels under reducing (lower panels) or nonreducing (upper panels) conditions. Thioredoxins were detected by Western blotting using specific antibodies at the following dilutions: anti-TrxA (α -TrxA), 1:1,000; α -TrxB, 1:10,000; α -TrxQ, 1:5,000.

Our previous screening of the *Synechocystis* cytosolic compartment for Trx-interacting proteins using a TrxA(C35S) site-directed mutant as the bait resulted in the identification of 1-Cys Prx and PrxII among the target proteins (32). The subsequent study, in which we compared the target proteomes of the three *Synechocystis* Trx, showed that 1-Cys Prx and PrxII, in addition to being targets for TrxA, are also targets for TrxQ and, to a lesser extent, for TrxB (48). In this study, we tested the abilities of all five Prx to interact with each of the three Trx. To this end, the recombinant Prx were incubated with the site-directed mutated versions of each Trx—TrxA(C35S), TrxB(C34S), and TrxQ(C33S)—thus obtaining stable mixed disulfides between the respective Trx and Prx when interactions did occur. Mixed disulfides were detected by Western blot analysis using antibodies raised against the respective Trx following electrophoretic separation under nonreducing conditions (Fig. 2). Since we have observed previously that *Synechocystis* Trx carrying site-directed mutations of their second active-site cysteines tend to form disulfide-bridged dimers and, in the case of TrxB(C34S), oligomers (48), the Trx were also incubated in the absence of Prx as a control in order to distinguish Trx dimers or oligomers from Prx/Trx adducts (Fig. 2). Incubation of the Prx with TrxA(C35S) followed by Western blotting using antibodies specific for TrxA showed that mixed disulfides were formed mainly between this Trx and 2-Cys Prx, 1-Cys Prx, and PrxQ1, though smaller amounts of mixed disulfides formed with PrxII and PrxQ2 were also detected (Fig. 2A, upper panel). As expected, electrophoresis under reducing

conditions abolished the observed Prx/Trx adducts, as detected by Western blotting (Fig. 2A, lower panel). The formation of disulfide-linked trimers and tetramers of TrxB(C34S) (Fig. 2B) due to the presence of a non-active-site cysteine in the amino acid sequence (48) made interpretation more difficult. However, bands with different migrations, indicating mixed disulfides, did appear for PrxII and PrxQ1 (Fig. 2B). It should be noted that the antibody raised against TrxB showed weak cross-reactivity against PrxII, PrxQ2, and PrxQ1 (Fig. 2B, lower panel). TrxQ(C33S) formed mixed disulfides with all Prx, particularly 1-Cys Prx, which in fact bound most of the added Trx (Fig. 2C). From this experiment it may be concluded that 2-Cys Prx is capable of interacting with TrxA and TrxQ; PrxII interacts with all Trx; 1-Cys Prx interacts strongly with TrxQ but also with TrxA; PrxQ2 interacts with TrxQ and weakly with TrxA; and PrxQ1 interacts moderately with all Trx.

Peroxidase activities of the *Synechocystis* Prx using the Trx as electron donors. Next, we explored the ability of the *Synechocystis* Prx to receive electrons and protons from each of the Trx in order to sustain peroxidase activity. To this end, equal amounts of the Prx were incubated with 4 μM each Trx reduced with a low (0.2 mM) concentration of DTT, and decomposition of H_2O_2 was measured (Fig. 3). DTT at this concentration did not produce appreciable rates of Prx activity in the absence of Trx (Fig. 3). Except for the couple TrxQ and PrxQ1, the *Synechocystis* Trx could indeed serve as electron donors for the entire complement of Prx from the same organism. The finding that nearly all Prx were able to receive electrons from

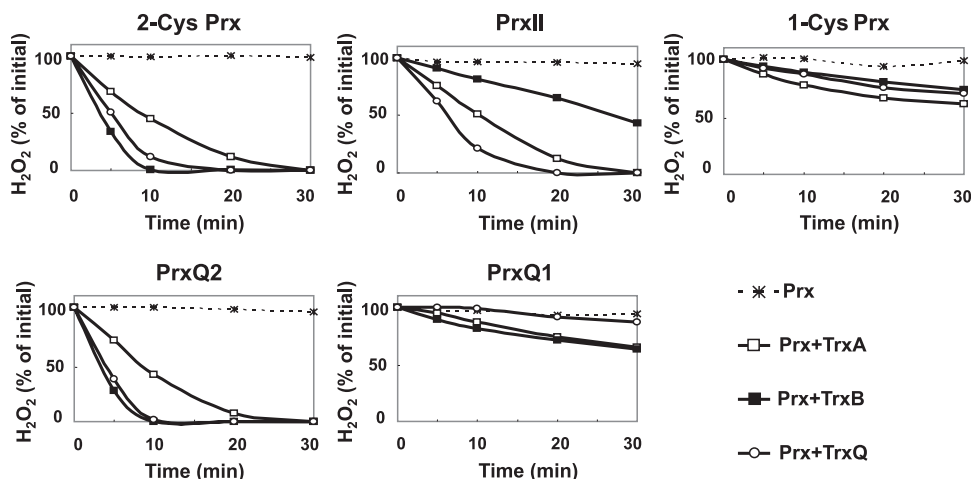


FIG. 3. Thioredoxin-dependent peroxidase activities of 2-Cys Prx, PrxII, 1-Cys Prx, PrxQ1, and PrxQ2. Reaction mixtures contained 50 mM HEPES-NaOH (pH 7.0), 0.2 mM DTT, 4 μ M Trx, 100 μ M H_2O_2 , and 25 μ g Prx in a final volume of 250 μ l. This concentration corresponds to 5 μ M for 2-Cys Prx, 1-Cys Prx, and PrxII and to 5.5 μ M for PrxQ1 and PrxQ2. The concentration of H_2O_2 was measured at 0, 10, 20, and 30 min after hydrogen peroxide addition. As a control, for each peroxidase an assay was performed in the absence of thioredoxins but in the presence of DTT (dashed lines). The values at each time point are averages from at least three independent experiments.

all Trx prompted a detailed analysis of the kinetic parameters in order to establish the possible preferences of each Prx for a particular Trx as the electron donor. To this end, Prx activities were measured at constant H_2O_2 concentrations (100 μ M), and the Trx concentrations were varied in the micromolar range for determinations of the apparent K_m values. The kinetic parameters presented in Table 2 allowed some conclusions to be drawn about catalytic efficiencies. First, TrxQ was the most efficient electron donor for 2-Cys Prx, PrxII, and PrxQ2, mainly due to the low apparent K_m values observed. Second, TrxB produced the highest reaction rates (k_{cat}) for 2-Cys Prx and PrxQ2, but these k_{cat} were counteracted by high apparent K_m values, and the catalytic efficiencies (k_{cat}/K_m) were fourfold lower than when TrxQ was used as the electron donor. Third, 1-Cys Prx and PrxQ1 displayed very low k_{cat} and k_{cat}/K_m irrespective of the Trx used to donate reducing equivalents; however, TrxA proved a more efficient electron donor for 1-Cys Prx than the other Trx, due to a low apparent K_m (Table 2).

The specificities of the *Synechocystis* Prx for peroxide substrates were examined by comparing the decomposition rates of hydrogen peroxide, *t*-BOOH, and CHP. Generally, the rates of decomposition of hydrogen peroxide and alkyl hydroperoxides were similar, except for PrxII, which decomposed CHP twice as fast as the other peroxides (Fig. 4).

Peroxidase activity of the *Synechocystis* Prx using GSH and Grx's as electron donors. To compare the efficiency of GSH as an electron donor for the five *Synechocystis* Prx, assays for peroxidase activity were performed using H_2O_2 as the substrate (Fig. 5A). Surprisingly, none of the Prx displayed peroxidase activity, as measured by the disappearance of peroxide, when supplied with GSH as the sole electron source (Fig. 5A). Assays for peroxidase activity were also performed using a system to regenerate GSH, consisting of glutathione reductase and NADPH, and the absorbance at 340 nm was monitored. However, no oxidation of NADPH above the background level occurred in the presence of Prx, demonstrating that GSH was

not consumed by the Prx (Fig. 5B). Since the cytosolic poplar PrxII has been shown to use Grx as an electron donor (56, 57), the His-tagged versions of *Synechocystis* GrxA and GrxB, encoded by the *ssr2061* and *slr1562* ORFs, respectively, were

TABLE 2. Kinetic parameters of the five different *Synechocystis* peroxidoredoxins using the three different thioredoxins as electron donors^a

Peroxidoredoxin and thioredoxin	K_m app (μ M)	V_{max} (nmol min ⁻¹ mg Prx ⁻¹)	k_{cat} (s ⁻¹) (10 ³)	k_{cat}/K_m (10 ³)
2-Cys Prx				
TrxA	9.6 \pm 1.8	237 \pm 38	92 \pm 1	10
TrxB	21.8 \pm 6.5	547 \pm 29	212 \pm 12	9.7
TrxQ	2.1 \pm 0.2	205 \pm 27	79 \pm 10	39
PrxII				
TrxA	4.6 \pm 0.8	273 \pm 34	99 \pm 10	22
TrxB	20.8 \pm 0.1	124 \pm 5	45 \pm 2	2
TrxQ	0.74 \pm 0.1	170 \pm 10	63 \pm 4	85
1-Cys Prx				
TrxA	0.9 \pm 0	25 \pm 1	10 \pm 0	11
TrxB	2.9 \pm 1.4	23 \pm 3	9 \pm 1	3
TrxQ	2.8 \pm 0.2	24 \pm 1	10 \pm 1	4
PrxQ2				
TrxA	12.2 \pm 3	269 \pm 65	78 \pm 10	6
TrxB	17.3 \pm 2.5	776 \pm 57	226 \pm 16	13
TrxQ	2.6 \pm 0.5	82 \pm 7	140 \pm 40	54
PrxQ1				
TrxA	6.6 \pm 3	48 \pm 14	15 \pm 4	2
TrxB	2.6 \pm 0.2	23 \pm 6	72 \pm 2	3
TrxQ				

^a Peroxidase activities were measured using 100 μ M hydrogen peroxide as the substrate. Each peroxidoredoxin was added at a final concentration of 10 μ M, except for PrxQ1 and PrxQ2, which were added at 11.5 μ M. Thioredoxin concentrations were varied from 0.1 to 20 μ M for determination of apparent K_m (K_m app) values. The data are means of the values obtained from three or four independent assays.

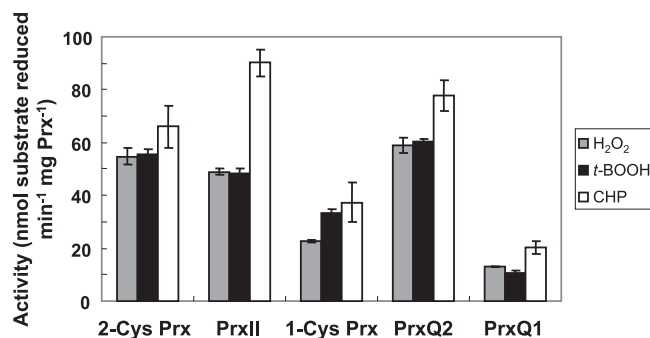


FIG. 4. Substrate specificities of 2-Cys Prx, PrxII, 1-Cys Prx, PrxQ1, and PrxQ2. Reaction mixtures contained 50 mM HEPES-NaOH (pH 7.0), 0.2 mM DTT, 4 μ M TrxA, 25 μ g Prx, and either 100 μ M H₂O₂, 100 μ M *t*-BOOH, or 50 μ M CHP in a final volume of 250 μ l. The reduction of peroxides was measured as described in Materials and Methods. The values at each time point are means from at least three independent experiments. Error bars, standard deviations.

expressed, purified, and examined for activity as electron donors for the five *Synechocystis* Prx (Fig. 5C and D). These experiments showed that both GrxA and GrxB possess some intrinsic peroxidase activity but are not able to donate elec-

trons to any of the Prx (Fig. 5C and D). The peroxidase activities of GrxA and GrxB were found to depend on the presence of either GSH or DTT (data not shown). Since our results were partially in disagreement with those of Hosoya-Matsuda et al. (2005) concerning PrxII, which was reported to use GSH rather than Trx as the electron donor (23), we also tested a peroxide concentration (250 μ M) higher than that used in their study. However, this only increased the nonenzymatic GSH-dependent background activity, and the presence of PrxII did not accelerate NADPH oxidation (see Fig. S1 in the supplemental material).

Expression of the *Synechocystis prx* and *trx* genes. Previous studies have shown that the expression of *prx* genes may differ in response to changes in growth conditions in plants as well as in cyanobacteria (8, 22, 63, 64). Little is known about *trx* gene expression in cyanobacteria, except that *Synechocystis trxA* transcription depends on the operation of photosynthetic or respiratory electron transport and that prolonged exposure of cultures to high light intensity results in a decrease in *trxA* transcript levels (41). Considering a possible correlation between the expression patterns of the *prx* and *trx* genes, we examined the transcript levels of all five *Synechocystis prx* genes and the three *trx* genes under various

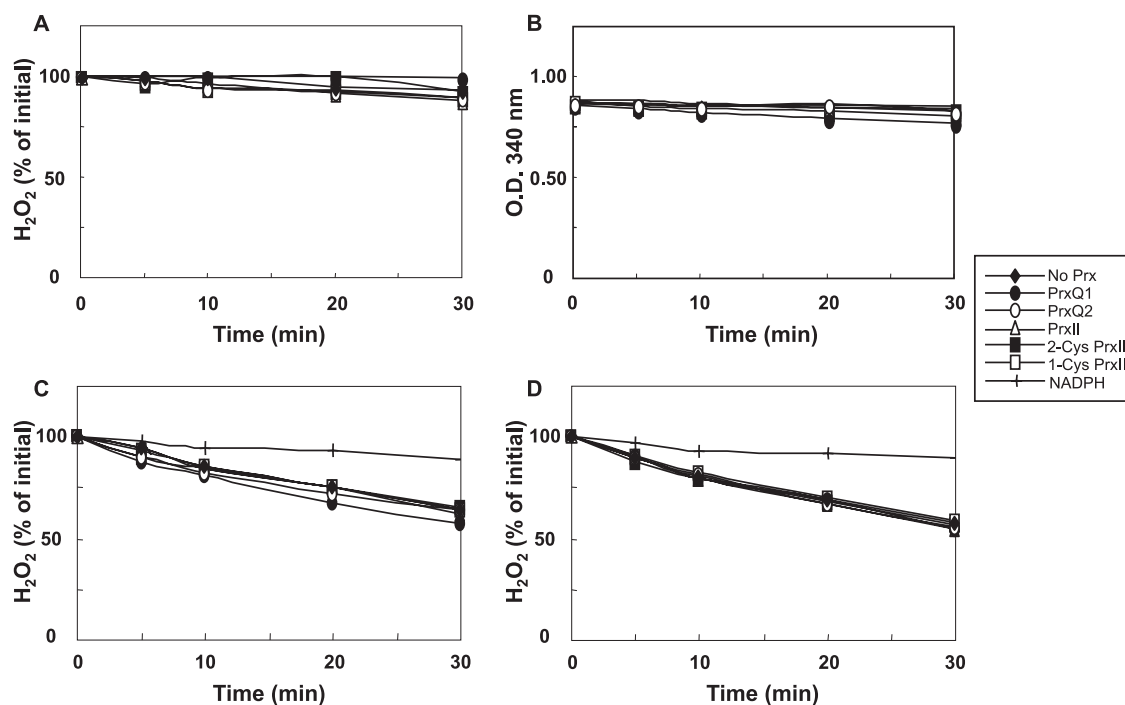


FIG. 5. Peroxidase activities of 2-Cys Prx, PrxII, 1-Cys Prx, PrxQ1, and PrxQ2 using GSH or *Synechocystis* glutaredoxins as electron donors. Each reaction mixture contained 75 mM potassium phosphate (pH 7.6), 100 μ M GSH, 100 μ M H₂O₂, and 25 μ g Prx in a final volume of 250 μ l. This concentration corresponds to 5 μ M for 2-Cys Prx, 1-Cys Prx, and PrxII and to 5.5 μ M for PrxQ1 and PrxQ2. (A) Reduction of hydrogen peroxide by peroxidases using GSH alone as the electron donor. Concentrations of hydrogen peroxide were determined by the FOX assay. (B) NADPH oxidation coupled to peroxidase activity using GSH as the electron donor and glutathione reductase-dependent regeneration. To this end, reaction mixtures were supplemented with 0.15 U glutathione reductase from yeast and 10 mM NADPH. NADPH oxidation was measured spectrophotometrically at 340 nm. (C and D) Decomposition of hydrogen peroxide using a GSH-glutaredoxin system as the electron donor for the peroxidases. Reaction mixtures were supplemented with 0.15 U glutathione reductase from yeast, 10 mM NADPH for regeneration, and either 4 μ M GrxA (C) or 4 μ M GrxB (D). Concentrations of hydrogen peroxide were determined by the FOX assay. Controls were performed with reaction mixtures containing no Prx but still containing the respective Grx (No Prx). Controls were also performed in the presence of NADPH, glutathione reductase, and GSH but without Grx and Prx (NADPH). The values at each time point are averages for at least three independent experiments.

conditions. To this end, Northern blot analyses were performed on RNA isolated from *Synechocystis* cultures following transfer to high light intensity, addition of H₂O₂ (Fig. 6), a temperature increase, or the removal of a nitrogen source from the medium (Fig. 7), and the relative mRNA levels were quantified.

Following the addition of 1 mM hydrogen peroxide to *Synechocystis* cultures, the transcript levels of the *trxA*, *trxB*, 2-*cys prx*, 1-*cys prx*, and *prxQ2* genes decreased during the first 15 min and thereafter increased to the initial levels (Fig. 6A and C). In contrast, the transcript levels of *trxQ*, *prxII*, and *prxQ1* showed a pronounced increase during the first 30 min; thereafter, they were reduced to the initial levels for *trxQ* and *prxII* but not for *prxQ1*, which remained upregulated (Fig. 6A and C). The expression of the *isiA* gene, which encodes an iron deficiency-related chlorophyll-binding protein (36), was monitored under the same conditions as a control, since expression of this gene is known to be induced by hydrogen peroxide (30, 62). The *isiA* gene was found to respond with a pattern similar to that of *trxQ*, *prxII*, and *prxQ1* (Fig. 6A).

Exposure to high light intensity resulted in enhanced transcription of several of the *prx* genes, and the most conspicuous response was observed for the *prxII* transcript, the level of which displayed a 10-fold increase (Fig. 6B and D). The expression of 1-*cys prx* appeared to be the least affected by high light conditions. Concerning *trx* expression, the *trxA* transcript and, to a greater extent, the *trxB* transcript accumulated continuously throughout the experiment, whereas the *trxQ* transcript level showed a transient increase during the first 30 min of high light (Fig. 6B and D). The *trxB* transcript exhibited the most dramatic change, with an increase of almost sixfold during the 5-h experiment (Fig. 6D). As a control, the *pgr5* transcript level was determined (Fig. 6B), since expression of this gene (*ssr2016*) has been shown to be enhanced following a shift from low to high light intensity (2).

Heat shock treatment produced no considerable changes in the expression of any of the *prx* genes, *trxA*, or *trxB*. In contrast, the *trxQ* transcript level increased about fourfold during the first hour of exposure to 43°C and thereafter dropped again (Fig. 7A and C). The expression of the *hspA* gene, which encodes the 16.6-kDa small heat shock protein (65), was examined as a positive control and was found to be induced more than 100-fold during the first 30 min (Fig. 7A).

Nitrogen starvation did not affect the expression of the *trx* genes significantly (Fig. 7B and D). In contrast to the other *prx* genes, the transcript level of the *prxII* gene increased throughout the experiment, whereas that of the 1-*cys prx* gene decreased sharply after 60 min. As expected, expression of the *glnN* gene, which encodes a glutamine synthetase (52), was rapidly induced by nitrogen starvation (Fig. 7B and D).

Thus, similar transcription patterns were observed for the *Synechocystis prxII*, *prxQ1*, and *trxQ* genes with respect to induction in response to H₂O₂. Common patterns were also detected for the 1-*cys prx* and *trxA* genes with respect to repression of transcription in response to H₂O₂. The 2-*cys prx* and *trxB* genes shared the common features of induction in response to high light intensity but not to H₂O₂.

DISCUSSION

Interactions between *Synechocystis Prx* and Trx. In this study an effort was made to provide an overview of the entire Prx complement from one oxygenic photosynthetic prokaryote, the cyanobacterium *Synechocystis*, with particular emphasis on the role of the Trx from this organism as electron donors to sustain Prx activity. To establish which Trx may interact with each Prx is of special relevance for a prokaryotic organism, where all these enzymes might reside in the same compartment. The only exception to the presumed cytosolic location is PrxQ2, which belongs to a phylogenetic subgroup of the cyanobacterial Prx Q homologues referred to as the GCT4 subcluster (8). Sequence analysis showed that all cyanobacterial Prx Q homologues from the GCT4 subcluster possess an N-terminal extension that is predicted to be a signal peptide with a probability close to unity (our unpublished data). Interestingly, the only *Arabidopsis* PrxQ was recently shown to be associated with chloroplast thylakoid membranes (28) and was further demonstrated to reside within the thylakoid lumen (50).

Since Prx enzymatic activity depends on the redox state of cysteines, resolution of Prx by protein gel electrophoresis is commonly performed both with and without disulfide reductants, such as DTT and β-mercaptoethanol. Under nonreducing conditions, the *Synechocystis* 2-Cys Prx migrated, as expected, as a dimer. This is a result of the disulfide formed during catalysis between the N-terminal peroxidatic cysteine from one subunit and the C-terminal resolving cysteine from another subunit, which is characteristic for typical 2-Cys Prx (72). Plant PrxII and Prx Q have been classified as atypical 2-Cys Prx, which means that the disulfides formed between peroxidatic and resolving cysteines are intramolecular (13, 55). Nevertheless, a fraction of *Synechocystis* PrxII, which conserves both cysteines with respect to plant PrxII isoforms, migrates as a dimer under nonreducing conditions (Fig. 1B). PrxQ2 migrated exclusively as a monomer in the absence of a reductant. This is in agreement with the study of Cha et al. (2007), which showed that all four Prx Q from *Anabaena* sp. strain PCC 7120 form intramolecular disulfides (8). *Synechocystis* PrxQ1 existed principally in the monomeric form under nonreducing conditions, but a minor fraction could also be observed as a dimer. This finding is somewhat unexpected, since the PrxQ1 homologue from *Anabaena* clearly is a monomeric enzyme (8). It is probably due to the fact that PrxQ1 contains a second cysteine 70 amino acids downstream from the peroxidatic cysteine, which apparently permits disulfide formation with another PrxQ1 molecule. Whether this disulfide is relevant for catalysis remains to be elucidated. The catalytic mechanism for 1-Cys Prx is still unknown (13), but the migration of *Synechocystis* 1-Cys Prx under nonreducing conditions (Fig. 1B) indicates that either of its two additional cysteines, apart from the conserved peroxidatic cysteine, may participate in intermolecular disulfide formation (23).

Plant Prx have been reported to be relatively slow peroxidases compared to ascorbate peroxidases and catalases (58). The peroxide decomposition rates measured for the *Synechocystis* Prx, using any of the three *Synechocystis* Trx as electron donors, lie within a k_{cat} range of 0.01 to 0.2 s⁻¹ (Table 2). These values are compatible with those observed for *Arabidopsis* 2-Cys Prx and Prx Q, which were reported to range from

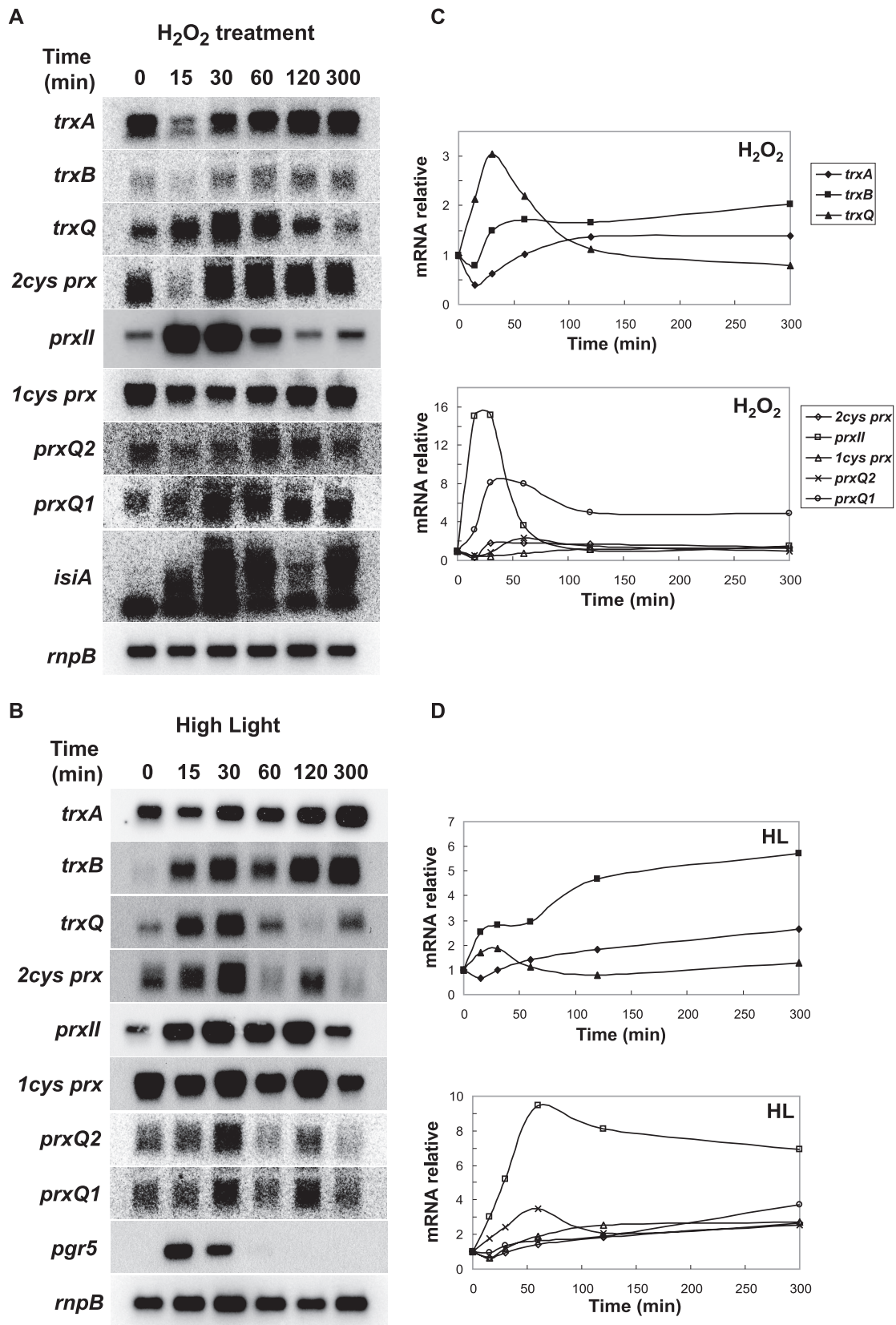


FIG. 6. Effects of hydrogen peroxide and high-light (HL) treatments on the expression of thioredoxin and peroxiredoxin genes. *Synechocystis* cells growing photoautotrophically were either treated with hydrogen peroxide (0.5 mM) (A and C) or exposed to a high light intensity ($500 \mu\text{E} \cdot \text{m}^{-2} \text{s}^{-1}$) (B and D). At the indicated times, samples were collected, and total RNA was obtained and hybridized with specific probes for each gene. Relative mRNA levels were determined and normalized to *rnpB* expression levels.

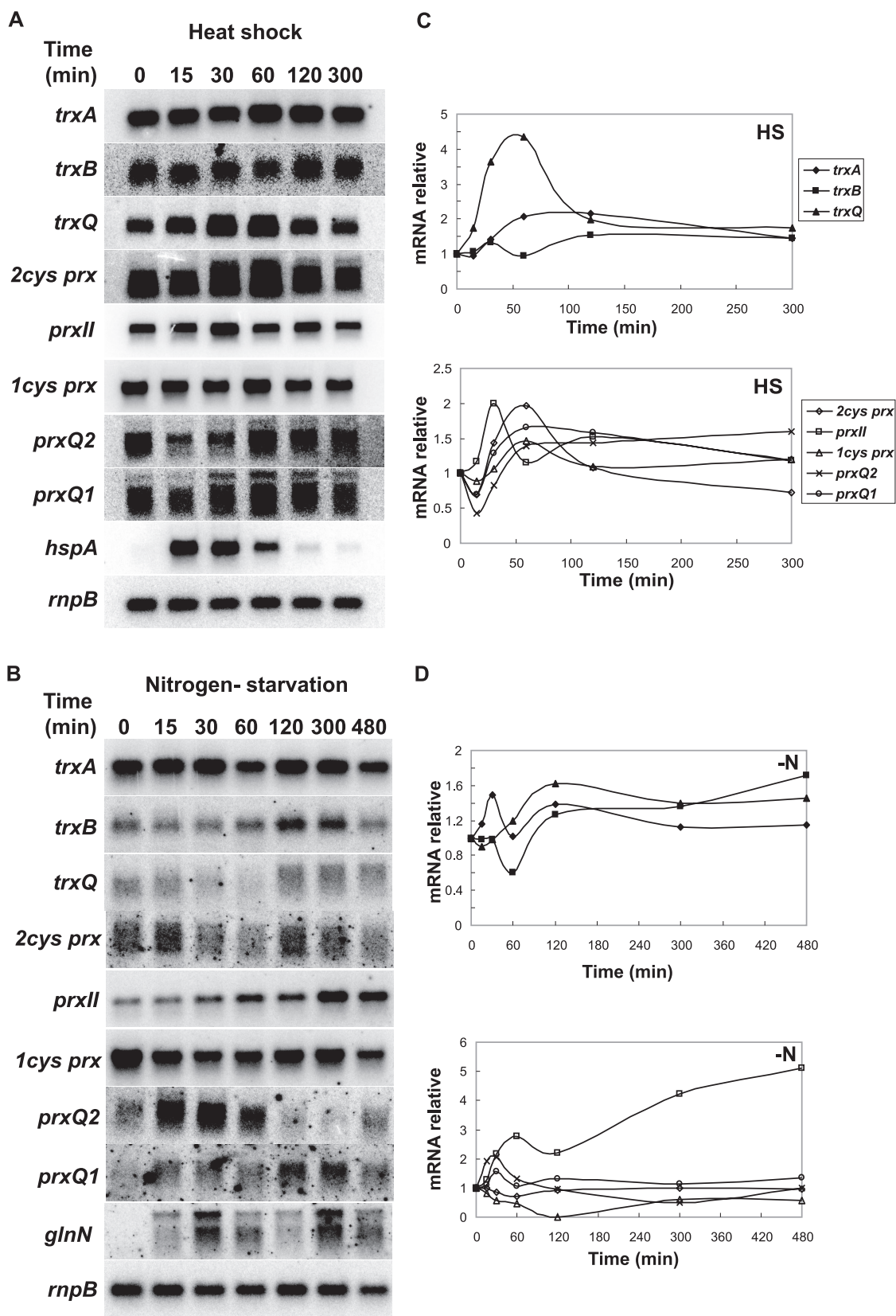


FIG. 7. Effects of heat shock (HS) and nitrogen deprivation (-N) on the expression of thioredoxin and peroxiredoxin genes. Either *Synechocystis* cells growing photoautotrophically were subjected to a heat shock treatment (43°C) (A and C) or the culture was transferred to a medium lacking a nitrogen source (B and D). At the indicated times, samples were collected, and total RNA was obtained and hybridized with the specific probes for each gene. Relative mRNA levels were determined and normalized to *rnpB* expression levels.

0.08 to 0.6 s⁻¹ when various chloroplast simple-module Trx were used as electron donors with DTT as the ultimate reductant of these Trx (9, 10). Notably, 100-fold-higher turnover numbers have been determined for the *Salmonella enterica* serovar Typhimurium 2-Cys Prx (AhpC) by using an assay that included a highly efficient reductant consisting of the *S. Typhimurium* AhpF N-terminal domain carrying an S128W substitution (44, 45). In the present study, however, we aimed at comparing the efficiencies and limitations of the endogenous *Synechocystis* Trx as electron donors for the Prx, and therefore, the assay developed in references 44 and 45 was not applicable.

Hosoya-Matsuda et al. (2005) have determined the peroxidase activities of the *Synechocystis* 1-Cys Prx and PrxII using the *Synechocystis* *x*-type Trx, which in our study is referred to as TrxB, as the electron donor (23). Using 0.5 mM H₂O₂ as the substrate, the measured maximal activities were 2.7 and 0.2 nmol per mg of protein per min for PrxII and 1-Cys Prx, respectively, considerably lower than the activities of all *Synechocystis* Prx determined in the present study, which range from 22 to 776 nmol per mg of protein per min (Table 2). However, Hosoya-Matsuda et al. (2005) used an *Arabidopsis* NTR as the electron donor for TrxB (23). Notably, the cyanobacterial *x*-type Trx was previously found to be unable to receive electrons from *E. coli* NTR (1), unlike the cyanobacterial *m*-type Trx (20, 31). Therefore, a possible explanation for the discrepancy between the activities observed in the present study (Fig. 3 and Table 2) and those observed in reference 23 is the choice of reductant of the Trx, since electron transfer between the NTR and the Trx may be rate limiting.

Previous biochemical characterization of plant chloroplast 2-Cys Prx and Prx Q, including assays of various chloroplast Trx together with DTT as electron donors (9, 10), allows a comparison with the data obtained from this study of their cyanobacterial homologues. It should be noted that in our assays we used 100 μM H₂O₂, whereas in references 9 and 10, 400 μM *t*-BOOH was used as the peroxide substrate. However, in all three studies, one could consider the peroxide concentration to be well above saturation. The *Arabidopsis* 2-Cys Prx was reduced in vitro by plastid simple-module Trx of the *m*, *f*, and *x* types, and the highest catalytic efficiency was obtained for Trx *x*, with a *k*_{cat} of 0.18 s⁻¹ and a *K*_m of 25 μM (9). These values are strikingly similar to those measured for *Synechocystis* 2-Cys Prx using the *x*-type Trx, TrxB, as the electron donor, which yielded a *k*_{cat} of 0.21 s⁻¹ and a *K*_m of 22 μM (Table 2). However, *Synechocystis* 2-Cys Prx could be considered most efficiently reduced by the *y*-type Trx, TrxQ, since the apparent *K*_m was as low as 2 μM (Table 2). The plastid Trx *y* isoforms had not yet been discovered at the time of the study by Collin et al. (2003) (9). Later, *Arabidopsis* Prx Q activity was found to be sustained in vitro by *Arabidopsis* Trx *y*1, Trx *y*2, Trx *x*, and Trx *m*4, and the highest catalytic efficiencies were measured for Trx *y*1 and Trx *y*2, which produced *k*_{cat} values of 0.29 and 0.56 s⁻¹ and *K*_m values of 1 and 2.6 μM, respectively (10). The *Synechocystis* *y*-type Trx, TrxQ, is also the best electron donor for PrxQ2, with a *k*_{cat} of 0.14 s⁻¹ and a *K*_m of 2.6 μM. However, it should be kept in mind that these activities may be physiologically irrelevant if the plant Prx Q is located in the thylakoid lumen (50) and the *Synechocystis* PrxQ2 is indeed luminal or periplasmic. Surprisingly, *Synechocystis* TrxQ proved completely inefficient toward PrxQ1, emphasizing that Prx Q rep-

resents a heterogeneous family in cyanobacteria (8; also this study).

The next issue to be addressed was whether there is a role for GSH or Grx as sources of reducing equivalents for cyanobacterial Prx, since, for example, a cytosolic poplar PrxII is able to use Grx as an electron donor (55–57). In contrast to the results in reference 23, where very high activities for *Synechocystis* PrxII were obtained using GSH with or without Grx, we observed no activity for any *Synechocystis* Prx by using GSH or Grx as the reducing agent. It should be noted that *Arabidopsis* chloroplast PrxII-E, one of the closest plant relatives of *Synechocystis* PrxII, could be reduced neither by GSH nor by chloroplast Grx (13). Furthermore, Tichy and Vermaas (1999) demonstrated that *Synechocystis* possesses no GSH-dependent peroxidase activity in vivo (69), which is also in agreement with the finding that the two glutathione peroxidase-like proteins encoded by the *Synechocystis* ORFs slr1171 and slr1992 are able to use NADPH, but not GSH, as an electron donor for the reduction of lipid hydroperoxides (16, 17). Concerning peroxide substrate specificity, we detected no significant preferences of any of the *Synechocystis* Prx, except for PrxII, which was markedly more active with the aromatic peroxide CHP. PrxII proteins from other photosynthetic organisms have not been studied previously in this regard, but poplar Prx Q decomposes H₂O₂, *t*-BOOH, and CHP, with a higher efficiency toward CHP (55), and *Arabidopsis* Prx Q reduces H₂O₂ and CHP at compatible rates but is less efficient toward *t*-BOOH and completely inefficient toward lipid hydroperoxides (28).

Expression of *prx* and *trx* genes under different stress conditions. Enhanced transcription of the *Synechocystis prxII* gene under high light conditions and in the presence of H₂O₂ is well documented. *Synechocystis* cDNA microarray analyses revealed that expression of the *prxII* gene was strongly upregulated by H₂O₂ (30) and methyl viologen, particularly under high light conditions (27). This was also observed in the study reported in reference 64, which confirmed the transient increase in *Synechocystis prxII* mRNA levels following treatment with either H₂O₂ or methyl viologen. Here we show a similar expression pattern for the *Synechocystis trxQ* gene following the addition of H₂O₂, which is consistent with the fourfold increase observed in a recent cDNA microarray analysis (25). The initial decrease in transcription of the *trxA* and *l-cys prx* genes during the first 30 min of peroxide treatment in our study is also in agreement with the values obtained from a cDNA microarray analysis (30), where these genes are reported to be repressed 2.1-fold and 2.0-fold, respectively, 30 min after addition of H₂O₂. Interestingly, global expression profiling of *Synechocystis* cells exposed to UV-B radiation displayed twofold and fourfold increases in the *trxQ* and *prxII* mRNA levels, respectively, whereas the *trxA* and *l-cys prx* transcript levels decreased threefold and twofold, respectively (24). Thus, there is a consistent pattern of upregulation of expression of the *prxII* and *trxQ* genes and downregulation of expression of the *l-cys prx* and *trxA* genes in response to H₂O₂ as well as to UV-B radiation.

In contrast to the transient nature of the responses to H₂O₂ addition, we found that nitrogen starvation triggered continuous induction of *prxII* transcription. Since nitrogen-containing macromolecules, such as proteins and DNA, represent major sinks for the primary products of photosynthesis, nitrogen de-

privation may lead to enhanced production of ROS (60), due to absorption of excess excitation energy even at moderate light intensities. Thus, it is likely that nitrogen starvation initiates continuous intracellular release of ROS, in contrast to the addition of exogenous H₂O₂ or methyl viologen, which results in a brief burst of intracellular ROS. In this context, it is worth mentioning that 1 mM H₂O₂ added to a culture of *Synechococcus* sp. strain PCC 7942 cells was decomposed within 15 min (46). Therefore, it is not surprising that no differences in the PrxII and 1-Cys Prx protein levels were detected during the hours following the addition of 0.5 mM H₂O₂ to *Synechocystis* cultures (23).

Unlike *prxII* and *trxQ*, the *Synechocystis* 2-cys *prx* gene did not respond appreciably to the addition of H₂O₂, though its transcription was induced by an increase in light intensity, suggesting that it is subject to a different control mechanism, independent of ROS. Previous studies have shown that illumination with high light intensity enhances transcription of both the *Synechocystis* 2-cys *prx* gene (64) and the *Synechococcus* sp. strain PCC 7942 2-cys *prx* gene (46). Among the *Synechocystis* *trx* genes, *trxB* represents the clearest example of induction under high light conditions but not in response to the addition of H₂O₂.

The *Synechocystis* *prxQ* genes were both induced by increased light intensity but responded differently to the addition of H₂O₂ and to nitrogen deficiency: *prxQ1* was transcribed more under the former condition and *prxQ2* was transcribed more under the latter. Stork et al. (2005) failed to detect any transcript of the *Synechocystis* *prxQ2* gene, referred to as *prxQ-B2* in their study, under any conditions (64). In the present study we clearly detected the *prxQ2* transcript in all Northern blot analyses, confirming that this gene is indeed expressed. In comparison, in plants the genes encoding the chloroplast PrxQ and PrxII proteins appear to be transcribed at higher levels under conditions that stimulate ROS production (50). Concerning the protein levels of the *Synechocystis* Prx, under standard growth conditions 1-Cys Prx and PrxII are highly abundant proteins, which are readily detectable on Coomassie-stained 2-dimensional electrophoresis gels (61) and are invariably found in the *Synechocystis* Trx target proteome (32, 34, 48). The 2-Cys Prx, PrxQ2, and PrxQ1 proteins have not been detected in proteomic studies (18, 61). As for Trx abundance, we have determined previously by quantitative Western blotting that the TrxA, TrxB, and TrxQ proteins constitute 0.25, 0.016, and 0.0046% of the total *Synechocystis* cellular protein, respectively (14), and in a different study, TrxA and TrxB were reported to constitute 1 and 0.25% of the total *Synechocystis* soluble protein, respectively (21). From experimental values of total cellular protein concentrations in *Synechocystis* cultures and volumes of cell pellets after centrifugation (data not shown), we estimate the intracellular protein concentration to be about 358 mg/ml. This would yield concentrations of 76, 4.7, and 1.4 μM for TrxA, TrxB, and TrxQ, respectively. Taking into account the kinetic parameters determined in this study, TrxA might be present at saturating concentrations for all Prx, whereas TrxB is saturating only for 1-Cys Prx and PrxQ1, and TrxQ is saturating only for PrxII.

We conclude that all *Synechocystis* Prx are expressed and are genuine Trx-dependent peroxidases and that GSH and Grx appear not to play roles in peroxide decomposition in this

organism. Taken together, our in vitro analyses of protein-protein interactions and enzymatic activities show that the *Synechocystis* Prx in general are able to interact with and receive electrons from the different Trx from the same organism. Hence, it is likely that in vivo, there is a high degree of functional overlap between the Trx with respect to reduction of these Prx. Here we show that the *Synechocystis* TrxQ is at least fourfold as efficient as an electron donor for PrxII, PrxQ2, and 2-Cys Prx as the other Trx and that, in contrast, TrxQ is completely inefficient toward PrxQ1. However, the 50-fold-higher abundance of TrxA (76 μM) than of TrxQ (1.4 μM) indicates that TrxA could be of relative importance as an electron donor to *Synechocystis* Prx, despite the superior catalytic efficiency of TrxQ. Since PrxII is one of the most abundant and, in addition, one of the most active *Synechocystis* Prx, it seems plausible that PrxII accounts for most of the Prx activity in this organism in vivo. As a result of the attempt to establish connections between individual Trx and Prx, we found that TrxQ and PrxII constitute the most remarkable couple, which stands out for its catalytic efficiency as measured by apparent k_{cat}/K_m , pronounced affinity, and similar expression patterns. The second couple worth noting is TrxA and 1-Cys Prx: they coincide in the unusual expression pattern of reduced transcription under conditions of elevated ROS concentrations, and TrxA is also the most efficient electron donor for *Synechocystis* 1-Cys Prx.

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