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phrA, the major photoreactivating factor in the cyanobacterium *Synechocystis* sp. strain PCC 6803 codes for a cyclobutane-pyrimidine-dimer-specific DNA photolyase

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Abstract A new broad-host-range plasmid, pSL1211, was constructed for the over-expression of genes in *Synechocystis* sp. strain PCC 6803. The plasmid was derived from RSF1010 and an *Escherichia coli* over-expression plasmid, pTrcHisC. Over-expressed protein is made with a removable N-terminal histidine tag. The plasmid was used to over-express the *phrA* gene and purify the gene product from *Synechocystis* sp. strain PCC 6803. PhrA is the major ultraviolet-light-resistant factor in the cyanobacterium. The purified PhrA protein exhibited an optical absorption spectrum similar to that of the cyclobutane pyrimidine dimer (CPD) DNA photolyase from *Synechococcus* sp. strain PCC 6301 (*Anacystis nidulans*). Mass spectrometry analysis of PhrA indicated that the protein contains 8-hydroxy-5-deazariboflavin and flavin adenine dinucleotide ($FADH_2$) as cofactors. PhrA repairs only cyclobutane pyrimidine dimer but not pyrimidine (6-4) pyrimidinone photoproducts. On the basis of these results, the PhrA protein is classified as a class I, HDF-type, CPD DNA photolyase.

Key words *Synechocystis* sp. strain PCC 6803 · *phrA* · DNA photolyase · Cyclobutane pyrimidine dimer · Over-expression plasmid · pSL1211

Abbreviations *CPD* Cyclobutane pyrimidine dimer · *HDF* 8-Hydroxy-5-deazariboflavin

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Introduction

Solar ultraviolet (UV) radiation places a severe stress on all photosynthetic organisms. The situation is worsened by depletion of the stratospheric ozone layer and the concomitant increase in solar UV (Madronich et al. 1998). In an attempt to understand the cellular processes important for UV protection in photosynthetic organisms, we chose the cyanobacterium *Synechocystis* sp. strain PCC 6803 as a model organism in which to study the role of enzymatic photoreactivation in the repair of UV-damaged DNA. In *Synechocystis* sp. strain PCC 6803, a single DNA photolyase homologue, PhrA, has been shown to account for the majority of UV resistance and photoreactivation (Ng and Pakrasi, submitted).

Data on the biochemical function of the PhrA protein are provided in this report and complement earlier genetic data on the role of *phrA* in *Synechocystis* sp. strain PCC 6803 (Ng and Pakrasi, submitted). DNA photolyase from *Synechococcus* sp. strain PCC 6301 (*Anacystis nidulans*) is known to contain 8-hydroxy-5-deazaflavin (HDF) (Eker et al. 1990), a cofactor that is not synthesized in *Escherichia coli* (Takao et al. 1989). DNA photolyases are present in very low abundance in most organisms that have been studied (Eker et al. 1990; Kim and Sancar 1993; Sancar and Rupert 1978). We therefore decided to construct an over-expression system for *Synechocystis* sp. strain PCC 6803 that would allow us to purify PhrA, complete with cofactors, directly and efficiently from the cyanobacterium.

A conditional over-expression vector, pFC1, has been described in *Synechocystis* sp. strain PCC 6803 (Mermet-Bouvier and Chauvat 1994). The plasmid was constructed from the λ phage promoter (p_R), the λcI857 repressor and the replicon from the broad-host-range plasmid RSF1010 (Scholz et al. 1989). The induction of transcription is achieved by shifting the temperature from 30 °C to 37–42 °C. Because of the potential instability of proteins at high temperature and difficulty with the subsequent purification of the over-expressed proteins, we chose to construct an alternative over-expression system. The rest of this re-

port describes the construction of such an over-expression vector and its successful application to the over-expression and purification of the PhrA protein. Using the purified PhrA, we were able to examine the cofactors and the enzymatic activity of the protein.

Materials and methods

Plasmids and bacterial strains

RSF1010 (Schweizer 1993) and pUCGM (Scholz et al. 1989) were provided by Dr. Michael Bagdasarian (Michigan State University, East Lansing, Mich.) and Dr. Herbert Schweizer (Colorado State University, Fort Collins, Colorado), respectively. pTrcHisC was obtained from Invitrogen (Carlsbad, Calif.).

All the *Synechocystis* sp. strain PCC 6803 strains used were derived from a glucose-tolerant isolate (Williams 1988). The *phrA* deletion mutant (T1011) was maintained in BG-11 supplemented with 20 μg spectinomycin dihydrochloride (Sigma, St. Louis, Mo.) ml^{-1}. Cells were grown under 40 μmol m^{-2} s^{-1} white light (General Electric cool white fluorescent lamps) at 30 °C. Any ultraviolet (UV) light was screened out with a plastic UV filter, UF5 (UV cutoff=383 nm; Cope Plastics, St. Louis, Mo.).

Escherichia coli strains XL1-Blue [F′::Tn*10 proA*$^+$*B*$^+$ *lacI*^{q} *Δ(lacZ)M15/recA1endA1 gyrA96* (Nal^r) *thi hsdR17*(r_k^-,m_k^+) *supE44 relA1 lac*] was used for the general propagation of plasmids. *E. coli* strain HB101 [F^- Δ(*gpt-proA*)62 *leuB6 supE44 ara-14 galK2 lacY1* Δ(*mcrC-mrr*) *rpsL20* (Str^r) *xyl-5 mtl-1 recA13*] was used in the triparental mating. The *E. coli* strains were grown in Luria-Bertani (LB) medium at 37 °C (Sambrook et al. 1989).

Construction of pSL1211

The *Synechocystis* sp. strain PCC 6803 over-expression plasmid was constructed from RSF1010, pTrcHisC and pUCGM in two cloning steps. First, the *Sma*I fragment (which contains the gentamycin acetyltransferase gene, *aacC1*) of pUCGM was cloned into the *Ssp*I site upstream of the *Ptrc* promoter in pTrcHisC. The resulting plasmid (pSL1204) was digested with *Sph*I and *Ssp*I. The 3.2-kb *Sph*I–*Ssp*I fragment was blunt-ended with T4 DNA polymerase (New England Biolabs, Beverly, Mass.) and then ligated to a 5.7-kb *Hinc*II fragment from RSF1010. This resulted in the plasmid pSL1211 (Fig. 1). pSL1211 was maintained in *E. coli* with 30 μg gentamycin sulfate (Sigma) ml^{-1}.

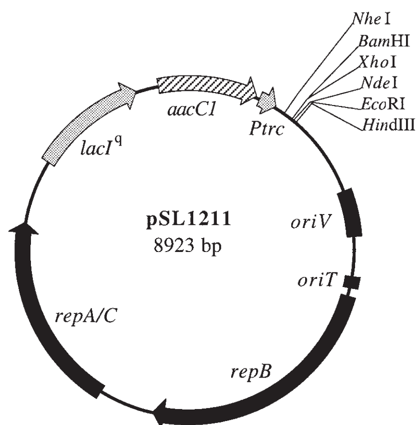

Fig. 1 Map of the broad-host-range over-expression plasmid pSL1211. A gentamycin acetyltransferase gene (*aacC1*), which confers resistance to gentamycin, is used as a selection marker. *repA, repB, repC* RSF1010 replication proteins, *oriV* RSF1010 origin of vegetative DNA replication, *oriT* RSF1010 origin of conjugational DNA transfer, *Ptrc trc* promoter

Conjugation

Triparental mating was conducted according to Elhai and Wolk (1988). Cells of *Synechocystis* sp. strain PCC 6803 and the *E. coli* strains SL1242 (*E. coli* HB101 cells tranformed with pSL1211) and RL443 (Elhai and Wolk 1988) were mixed and allowed to mate on a nitrocellulose membrane on solid BG-11 medium for 12 h under 40 μmol m^{-2} s^{-1} white light (General Electric cool white fluorescent lamps) at 30 °C. Exconjugants were selected by transferring the filter to solid BG-11 medium supplemented with 4 μg gentamycin sulfate ml^{-1}. Exconjugants appeared 4–5 days after conjugation. Since *E. coli* cannot grow on BG-11, there is no need to purify the exconjugants away from *E. coli* cells. The resulting exconjugants of *Synechocystis* sp. strain PCC 6803 carrying pSL1211 were maintained in BG-11 medium containing up to 30 μg gentamycin sulfate ml^{-1}.

Total DNA was purified from *Synechocystis* sp. strain PCC 6803 exconjugants carrying pSL1211, and *E. coli* XL1-Blue cells were transformed with the total DNA preparation. Gentamycin-resistant *E. coli* colonies were selected. Plasmid preparations from these clones were digested with restriction enzymes to check the identity of the plasmid and its stability in *Synechocystis* sp. strain PCC 6803.

Construction of the PhrA over-expression plasmid

The *phrA* gene from *Synechocystis* sp. strain PCC 6803 was excised from pSL1055 (Ng and Pakrasi, submitted) with *Bam*HI and *Hin*dIII. This *Bam*HI–*Hin*dIII fragment was sub-cloned into the *Bam*HI and *Hin*dIII sites of pSL1211, resulting in the plasmid pSL1276. This plasmid was conjugated into the *phrA* deletion strain of *Synechocystis* sp. strain PCC 6803 (T1011) by triparental mating.

Quantification of plasmid copy number

A filter hybridization assay (Shields et al. 1986) was used to examine the copy number of pSL1211 in *Synechocystis* sp. PCC 6803. Wild-type cells harboring the plasmid pSL1211 (T1242) were used to determine the copy number of pSL1211. Wild-type cells without the plasmid and the *phrA* deletion mutant cells were used as controls for hybridization. Exponential phase *Synechocystis* sp. strain PCC 6803 cells were harvested and resuspended in TE buffer (pH 8) supplemented with 0.02% sodium azide. The cell suspensions were kept on ice until used. Cells were broken three times with a French pressure cell press at 6.9 mPa (Spectronics Instruments, Rochester, N.Y.). Cell counts were made before and after the French press treatment using a Petrohoff-Hauser chamber. About 98% of the cells were broken; only the portion of cells that were broken was used to compute the final copy number of the plasmid. Crude cell extracts were serially diluted and denatured in 0.4 N sodium hydroxide. The dilutions were applied to two nylon membranes in duplicate by a dot-blotting device (Bio-Rad, Hercules, Calif.). Purified pSL1276 plasmid ($A_{260/280}$=1.76) was also blotted and served as the DNA standard. One nylon membrane was hybridized to a ^{32}P-labeled *phrA*-specific probe and the other was hybridized to a ^{32}P-labeled pSL1211-specific probe. Radioactivity on the blots was visualized with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.). The PhosphorImager signals were quantified with the ImageQuant software (Molecular Dynamics).

Over-expression and purification of PhrA

PhrA was purified from the *phrA* deletion mutant carrying the PhrA over-expression plasmid (strain T1011–1276). The culture

was grown to exponential phase and transcription was induced with 0.1 μM isopropyl-β-D-thiogalactopyranoside (IPTG) for 24 h. Cells were harvested by centrifugation and resuspended in a lysis buffer (50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl, 10 mM imidazole) supplemented with a 1:1,000 dilution of a general protease inhibitor cocktail (Sigma, catalog no. P2714). An equal volume of sterile glass beads (0.10–0.11 mm) was added and the mixture was vortex-mixed for 30 s and then placed on ice for 30 s. The vortexing was repeated 20 times. The glass beads and cell debris were removed by centrifugation at 4,000×*g*. The pellet was washed twice with a total of two volumes of lysis buffer. The combined supernatant was subjected to ultra-centrifugation in a Beckman Ultracentrifuge TL-100 at 137,000×*g* for 40 min to remove the thylakoid and cell membranes. The supernatant was removed and purified using a Ni-NTA resin (QIAGEN, Valencia, Calif.) under native conditions according to the manufacturer's instructions. PhrA has a distinct yellow color and its elution from the Ni-NTA column was monitored visually. The purified protein was dialyzed against several changes of a DNA photolyase buffer (40 mM NaCl, 10 mM potassium phosphate, 5 mM 2-mercaptoethanol, pH 7.0) (Eker et al. 1990). The protein sample was stored at –20 °C in 25% glycerol until use.

For SDS-PAGE, protein samples were mixed with equal volumes of loading buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, 10% mercaptoethanol, pH 6.8) and denatured at 75 °C for 5 min. The samples were then separated on a 10% polyacrylamide gel. To visualize the protein bands, the gel was stained with Coomassie brilliant blue R-250. For Western blotting, proteins on the gel were transferred to nitrocellulose membrane using a Trans-Blot SD Semi-dry Transfer Cell (Bio-Rad). The Western analysis was conducted as described in Durrant and Fowler (1994). The *phrA* gene was over-expressed in *E. coli* strain XL1-Blue with a N-terminal histidine tag using the plasmid pTrcHisC (Invitrogen). This histidine-tagged PhrA protein was purified with the Ni-NTA resin (QIAGEN) under denatured conditions according to the manufacturer's instructions. A rabbit antiserum raised against this denatured PhrA protein (Cocalico Biologicals, Reamstown, Penn.) was used as the primary antibody. A goat anti-IgG–horseradish peroxidase conjugate (Pierce, Rockford, Ill.) was used as the secondary antibody. The Western blot was developed with the SuperSignal Chemiluminescent Substrate (Pierce, Rockford, Ill.).

Absorption spectrum and mass spectrometry

PhrA was diluted in the DNA photolyase buffer (described above) and the absorption spectrum was recorded on a DW-2000 UV-VIS spectrophotometer (SLM Instruments, Urbana, Ill.) with a slit width of 1 nm.

The MALDI-TOF (matrix-assisted laser desorption/ionization-time-of-flight) experiments were carried out on a Voyager DE-RP instrument (Perseptive Biosystems, Framingham, Mass.). A saturated solution of sinapinic acid (Fluka, Milwaukee, Wis.) in a 2:1 ratio (v/v) of acetonitrile and H_2O was used as the matrix. Samples were dissolved in H_2O, and a 0.5-μl sample aliquot (1.5 μg PhrA) and 0.5 μl of the matrix solution were mixed on a stainless steel plate. Samples were desorbed as positive ions in the linear mode with a N_2 laser (337 nm) and accelerated with a potential of 25 kV. Data were collected with a Tektronics TDS 520A digitizing oscilloscope (Tektronics, Beaverton, Ore.) and processed with the Perseptive Grams/386 software, version 3.04 (Perseptive Biosystems). The MALDI spectra were obtained from approximately 20 laser shots and were signal-averaged. Mass calibration was done using the Sequazyme Peptide Mass Standards Kit (Perseptive Biosystems).

DNA photolyase assay

Synthetic DNA oligodeoxynucleotides containing centrally located, site-specific DNA photoproducts (Smith and Taylor 1993) were used to assess the DNA photolyase activity and substrate specificity of PhrA. Two types of photoproducts, the *cis-syn* cyclobutane pyrimidine dimer (C-S dimer) and the pyrimidine (6–4) pyrimidinone photoproduct (6–4 photoproduct), were tested as potential substrates for PhrA. The schematic representation of the assay is presented in Fig. 5A. The photoproduct-containing substrates were prepared as described in Reardon et al. (1993). The photoproduct-containing oligodeoxynucleotides were end-labeled with γ[^{32}P]ATP using T4 polynuclotide kinase (New England Biolabs, Beverly, Mass.) and then annealed to the complementary strand. The labeled substrates and PhrA were then incubated in DNA photolyase buffer in the light (General Electric cool white fluorescent lamp; 50 μmol m^{-2} s^{-1}) or in the dark for 2 h at room temperature (24–25 °C). Because direct reversal of the DNA dimers by DNA photolyase restores the unique *Mse*I site in the oligodeoxynucleotides, photorepair can be followed indirectly by *Mse*I cleavage. If photorepair occurs, 21-mer nucleotides will be released upon digestion with *Mse*I. DNA fragments were separated in a 10% glycerol-tolerant (0.09 M Tris-base, 0.29 M taurine, 0.02% (w/v) Na_2EDTA) polyacrylamide gel. Radioactivity on the gel was visualized with a PhosphorImager (Molecular Dynamics).

Results

Over-expression vector

The plasmid pSL1211 (Fig. 1) consists of three major components. The over-expression module is derived from the plasmid pTrcHisC (Invitrogen). It includes the *Ptrc* promoter, *lac* operator, and other downstream transcriptional and translational elements. The protein is made as a fusion product with a removable N-terminal histidine tag. In addition, the *lacI*^{q} repressor in the plasmid allows transcriptional regulation of cloned genes by the addition of IPTG. The selection marker, *aacC1* (which encodes for a gentamycin acetyltransferase), is derived from the plasmid pUCGM (Schweizer 1993). It confers antibiotic resistance to gentamycin (up to 30 μg gentamycin sulfate ml^{-1} in *Synechocystis* sp. strain PCC 6803 cells). The final component of the plasmid is derived from the broad-host-range plasmid RSF1010 (Scholz et al. 1989). It contains the origin of vegetative DNA replication (*oriV*) and the genes encoding all the replication proteins (*repA*, *repB* and *repC*) (Haring and Scherzinger 1989). These elements allow RSF1010 to replicate in over 30 species of gram-negative bacteria (Frey and Bagdasarian 1989). Furthermore, the origin of conjugal transfer (*oriT*) and the genes encoding the plasmid mobilization proteins (*mobA*, *mobB*, and *mobC*) (not shown in Fig. 1) from RSF1010 are also included in pSL1211. As a result, pSL1211 is self-mobilizable for conjugation.

The copy number of pSL1211 in *Synechocystis* sp. strain PCC 6803 was examined with a dot-blot filter hybridization assay. Owing to the tough cell envelopes of *Synechocystis* sp. strain PCC 6803, the cells cannot be lysed directly on a nylon filter. Instead, they were ruptured with a French press (with about 98% efficiency). The cell lysate was treated with 0.4 N NaOH and then applied to nylon filters. The radioactive probes hybridized to their respective target sequences with high specificity. The pSL1211-specific and the *phrA*-specific probes did not cross-hybridize to the *Synechocystis* sp. strain PCC 6803 wild-type and the *phrA* deletion mutant (T1011) cell lysates, respectively (results not shown). The purified

phrA over-expression plasmid (pSL1276) was used as a standard to compare the concentration of DNA in cell lysates, since it contains both the pSL1211-specific and the *phrA*-specific elements. In exponential phase *Synechocystis* sp. strain PCC 6803 cells, there was an average of 29 copies of pSL1211 per cell, equivalent to about 3.3 copies per chromosome (data not shown). The copy number of pSL1211 is about three times that of another RSF1010-derived plasmid, pSB2A (10 copies/cell), in *Synechocystis* sp. strain PCC 6803 (Marraccini et al. 1993). In *E. coli*, the copy number of RSF1010 per chromosome was estimated to be 10 (Frey and Bagdasarian 1989).

Over-expression and purification of PhrA

The *phrA* over-expression plasmid (pSL1276) was introduced to the *Synechocystis* sp. strain PCC 6803 *phrA* deletion mutant (T1011) by triparental mating. All of the eight exconjugants examined contained plasmids of the original size. Based on the restriction pattern of the recovered plasmids, there was no apparent rearrangement of the plasmid (data not shown).

On the basis of Western blot analysis, optimal conditions for the production of PhrA were 0.1 mM IPTG and 24 h of exposure (data not shown); these conditions were used for over-expression of PhrA. Using the Ni-NTA resin affinity chromatography, the histidine-tagged PhrA protein [$(His)_6$-PhrA] was isolated in high purity in one step (Fig. 2A). The purified $(His)_6$-PhrA fusion protein has an apparent molecular mass of 58 kDa and constitutes about 0.6% of the total protein. This $(His)_6$-PhrA protein will be referred to below as PhrA. Due to the low abundance of the protein, we were unable to detect PhrA in wild-type cells by Western blot analysis (Fig. 2B; detection limit of the Western blot analysis is 11.2 ng PhrA). We were able to detect PhrA only when the over-expression system was used (Fig. 2B, lane Δ*phrA*-pSL1276). The signals between 60 and 70 kDa were unspecific and were present in all lanes, including the *phrA* deletion mutant lane. Since the *Ptrc* promoter that was used to drive transcription is leaky, the PhrA protein was detected in both induced and uninduced *Synechocystis* sp. strain PCC 6803 cells (data not shown). As a result, uninduced cells were not included as a control.

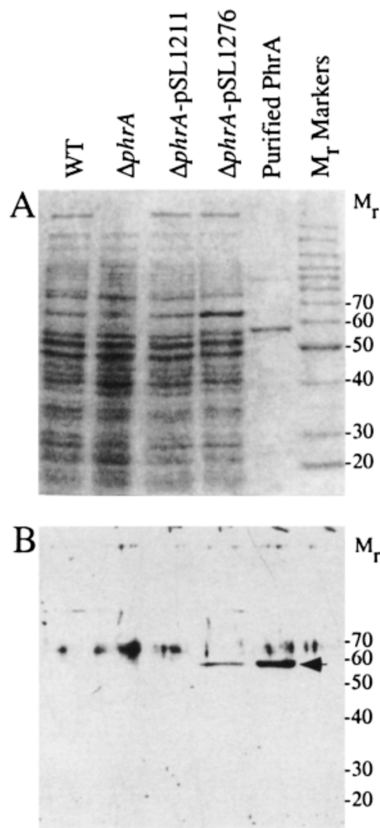

Fig. 2A,B Purification of PhrA from *Synechocystis* sp. strain PCC 6803. The $(His)_6$-PhrA was purified from the *Synechocystis* sp. strain PCC 6803 strain carrying the PhrA over-expression plasmid (*phrA*-pSL1276) using Ni-NTA resin. **A** SDS-PAGE of soluble protein extracts stained with Coomassie brilliant blue R-250. **B** Corresponding Western blot of the SDS-polyacrylamide gel. The Western blot was probed with a PhrA-specific antiserum, as described in Materials and methods. The *arrow* indicates the $(His)_6$-PhrA bands (apparent molecular mass: 58 kDa). Native PhrA has a molecular mass of 56.03 kDa. *WT*, Wild type; Δ*phrA, phrA* deletion strain; Δ*phrA*-pSL1211, pSL1211 in the *phrA* deletion strain; Δ*phrA*-pSL1276, the PhrA over-expression plasmid pSL1276 in the *phrA* deletion strain

Optical absorption spectrum of PhrA

Purified PhrA protein has a pale yellow color. Its optical absorption spectrum is shown in Fig. 3 and closely resembles that of *Synechococcus* sp. strain PCC 6301 (*Anacystis nidulans*) DNA photolyase (Eker et al. 1990). Like the absorption spectrum of *Synechococcus* sp. strain PCC 6301 DNA photolyase, the PhrA spectrum has two prominent absorption peaks, at 273 nm and 442 nm. Compared to the *Synechococcus* sp. strain 6301 DNA photolyase, which has a peak at 437 nm, the visible absorption peak of *Synechocystis* sp. strain PCC 6803 PhrA is red-shifted by 5 nm. Another notable difference between the two spectra is the presence of a small shoulder at 422 nm in the PhrA

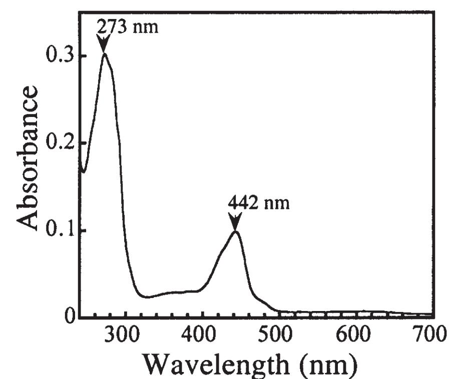

Fig. 3 Optical absorption spectrum of PhrA at room temperature . Absorption maxima are 273 nm and 442 nm

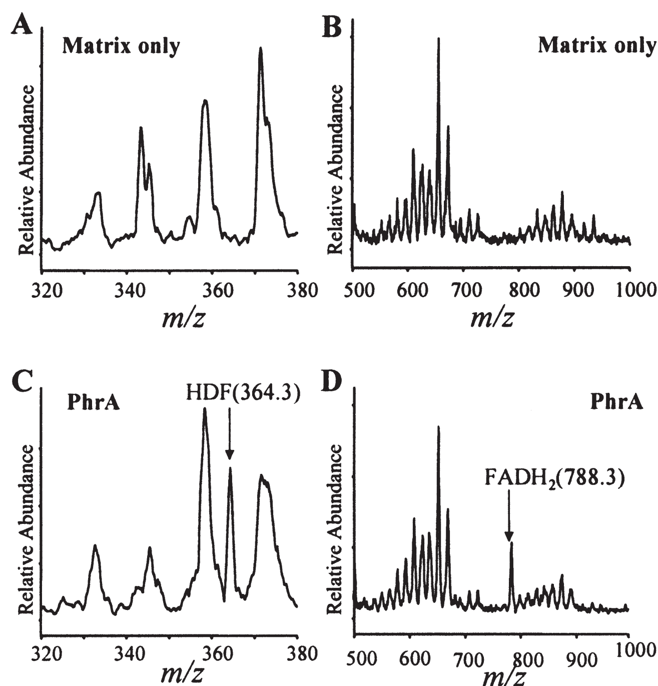

Fig. 4A–D Identification of PhrA cofactors by MALDI-TOF mass spectrometry. **A, B** Positive-ion spectra of matrix only. **C, D** Positive-ion spectra of PhrA in matrix. *Arrows* indicate the peaks of $[M+H]^+$ ions of the two cofactors. The corresponding *m/z* values are indicated in *parentheses*. Calculated molecular weights of HDF and $FADH_2$ are 363.3 and 787.6, respectively

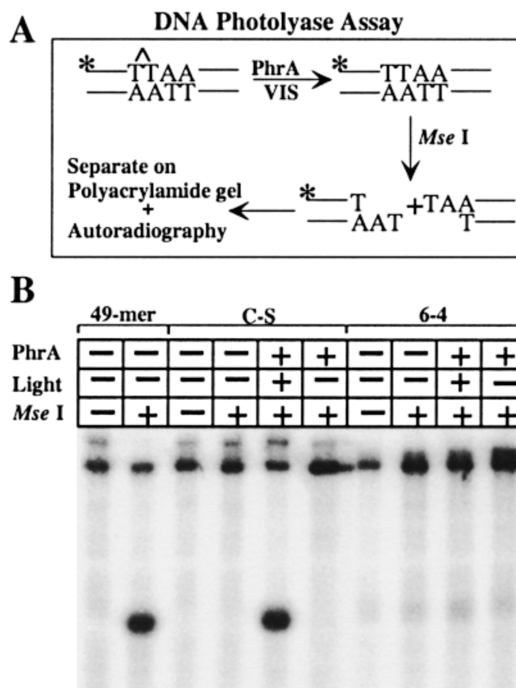

Fig. 5A, B DNA photolyase assay for PhrA. Oligodeoxynucleotides containing either the *cis-syn* (C-S) cyclobutane pyrimidine dimer or pyrimidine (6-4) pyrimidinone photoproduct were used to test the DNA photolyase activity and substrate specificity of PhrA. **A** Schematic representation of the DNA photolyase assay; **B** DNA photolyase assay. The experiment was carried out as described in Materials and methods. A 49-mer duplex without any photoproduct was used as a control for the *Mse*I digestion

spectrum, which is absent in the spectrum of *Synechococcus* sp. strain 6301 DNA photolyase.

Mass spectrometry

One of the most distinct biochemical characteristics of DNA photolyases is the presence of two non-covalently bound cofactors. MALDI-TOF mass spectrometry was used to examine the cofactors associated with PhrA (Fig. 4). Panels A and B in Fig. 4 show the mass spectra of the matrix without any PhrA sample. The peaks in these two spectra came from the matrix. With the addition of purified PhrA, two new and distinct peaks are observed at *m/z* 364.3 and 788.3. The peak at *m/z* 364.3 corresponds to the $[M+H]^+$ ion of HDF (calculated molecular weight: 363.3) (Fig. 4C). The peak at *m/z* 788.3 corresponds to the $[M+H]^+$ ion of flavin adenine dinucleotide ($FADH_2$) (calculated molecular weight: 787.6; Fig. 4D). These results support the conclusion that HDF and $FADH_2$ are cofactors of the PhrA protein in vivo.

DNA photolyase assay

Since PhrA possesses all the biochemical signatures of a DNA photolyase, we examined the enzymatic activity of the protein towards the two most abundant photoproducts induced by ultraviolet light, the *cis-syn* cyclobutane pyrimidine dimer (*cis-syn* CPD) and 6-4 photoproduct (Fig. 5B). The 49-nucleotide band (Fig. 5, lane 1) comes from the original substrate and the 21-nucleotide band is the product of the *MseI* digestion (Fig. 5, lane 2). The bands above the 49-nucleotide bands (lanes 1, 3–6) may be due to incomplete denaturation of the DNA duplex or incomplete inactivation of the T4 polynucleotide kinase, which led to labeling of the complementary strand. PhrA exhibited significant light-dependent repair activity towards the *cis-syn* CPD (lanes 4, 5). There was no spontaneous repair in the dark (lane 3) or light (lane 4) in the absence of PhrA. The remaining 49-nucleotide band could be due to the unrepaired DNA duplex or single-stranded oligonucleotides that were never annealed to the complementary strand. PhrA has no detectable DNA photolyase activity towards the 6–4 photoproduct in the light or dark (lanes 7–10). The faint signals around the 21-nucleotide position are present in all four lanes and are probably due to the degradation of the 6-4 photoproduct-containing oligodeoxynucleotide.

Discussion

We have successfully applied the over-expression plasmid pSL1211 to production of the PhrA protein. The *Ptrc* promoter appeared to be functional in the *Synechocystis* sp.

strain PCC 6803 cells. Under optimal conditions, the over-expressed PhrA protein constituted about 0.6% of the total protein. In our experience, this yield of over-expressed protein is about ten times lower than what we can obtain with the *E. coli* over-expression system. It awaits further experimentation to elucidate whether this low level of expression is due to the low copy number of the plasmid, the suboptimal activity of the *Ptrc* promoter in a heterologous host, the low stability of the PhrA protein or other post-transcriptional problems. Further refinements in the transcriptional and translational elements are needed to provide a high-yield system. Also, increasing the copy number of the plasmid can augment the protein over-expression process by increasing the gene dosage.

Our previous study has shown that the *phrA* gene is the major UV resistance factor in *Synechocystis* sp. strain PCC 6803 (Ng and Pakrasi, submitted). Mutation in *phrA* renders cells hypersensitive to both UVB (280–320 nm) and UVC (200–280 nm). Based on the high sequence similarity of PhrA to the CPD DNA photolyase from *Synechococcus* sp. strain 6301 (including most of the cofactor binding sites), we predicted that PhrA is a CPD photolyase with HDF and $FADH_2$ as cofactors (Ng and Pakrasi, submitted). Here we have provided the biochemical evidence to support these assertions. Using synthetic, photoproduct-containing oligodeoxynucleotide substrates, we have conclusively shown that PhrA is a CPD-specific DNA photolyase. Mass spectrometry analysis identified two small molecules associated with the PhrA sample that correspond to the molecular masses of HDF and $FADH_2$. We therefore confirmed that PhrA contains the same cofactors as in the *Synechococcus* sp. strain 6301 DNA photolyase. The red-shift of the visible light absorption peak in PhrA is probably due to the different protein environment surrounding HDF in PhrA.

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