UV-A-induced expression of GroEL in the UV-A-resistant marine cyanobacterium *Oscillatoria* sp. NKBG 091600

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The authors have examined the response to UV-A irradiation of the UV-A-resistant marine cyanobacterium *Oscillatoria* sp. NKBG 091600, which produces the UV-A-absorbing compound biopterin glucoside. The expression of a 60 kDa protein was markedly induced at 500 min after UV-A irradiation. This protein was identified by N-terminal amino acid sequence analysis as GroEL. Northern blot analysis demonstrated that GroEL synthesis was controlled by UV-A at the transcriptional level. A CIRCE element and a putative SOS consensus sequence were found upstream of the *groESL* operon, overlapping two putative promoter sequences. Primer extension analysis revealed that *groESL* transcription in UV-A-induced cells starts from the proximal promoter overlapped by the SOS consensus sequence. This indicates that an SOS response regulation is instrumental in UV-A-induced GroEL expression of *Oscillatoria* sp. NKBG 091600. Furthermore, this UV-A-inducible GroEL may function to upregulate biopterin glucoside biosynthesis, thereby allowing growth under UV-A irradiation.

Keywords: stress response, UV-A resistance, biopterin glucoside, GroEL, marine cyanobacterium *Oscillatoria* sp.

INTRODUCTION

UV-A irradiation causes oxidative stress in several eukaryotes and prokaryotes (Bose *et al.*, 1990; Cadet *et al.*, 1993; Hu & Tappel, 1992; Parat *et al.*, 1995; Savoure *et al.*, 1996; Yamada *et al.*, 1996). UV-absorbants have been found in several cyanobacteria (Matsunaga *et al.*, 1993; Scherer *et al.*, 1988; Shibata, 1969), and the biosynthesis of these pigments was found to be UV-responsive (Ehling-Schulz *et al.*, 1997; Matsunaga *et al.*, 1993; Scherer *et al.*, 1988). We have investigated the physiological response of a UV-A-resistant marine cyanobacterium, *Oscillatoria* sp. NKBG 091600, to UV-A irradiation (Matsunaga *et al.*, 1993). A UV-A-absorbing pigment was isolated and identified as biopterin glucoside, which is chemically related to pterin pigments of butterfly wings. UV-A was found to be a potent elicitor of biopterin glucoside

biosynthesis. UV-A may cause significant damage to cyanobacteria by inhibiting energy acquisition by the photosynthetic reaction system (Hirose & Miyachi, 1982, 1983; Sinha *et al.*, 1995). Wachi *et al.* (1995) investigated the effect of UV-A intensity on cell growth, biosynthesis of biopterin glucoside and photosynthetic activity in a UV-A-resistant *Oscillatoria* sp., and observed that the loss of photosynthetic activity following exposure to UV-A was attenuated by prior UV-A exposure. These observations support the hypothesis that biosynthesis of UV-absorbing pigments functions as an adaptive strategy for protection against the adverse effects of UV irradiation. To further elucidate the biochemical basis of cyanobacterial UV-A resistance, we have examined the effect of UV-A irradiation on protein synthesis in *Oscillatoria* sp. NKBG 091600.

METHODS

Cyanobacterial strains and culture conditions. The marine *Oscillatoria* sp. NKBG 091600 and the freshwater *Synechococcus* sp. PCC 7942 were used in this study. NKBG 091600 and PCC 7942 were cultured in BG-11 medium (Allen & Stanier, 1968), supplemented with and without 3% NaCl,

Abbreviations: CIRCE, controlling inverted repeat of chaperone expression; IR, inverted repeat.

The GenBank accession number for the sequence reported in this paper is AF054630.

respectively, under cool-white fluorescent light ($500 \mu\text{W cm}^{-2}$) at 28°C . Initial cell density was 10^6 cells ml^{-1} . Growth was monitored by measuring OD_{750} .

UV-A treatment of cyanobacterial cells. Cells grown to mid-exponential phase with $500 \mu\text{W cm}^{-2}$ of cool-white fluorescent light irradiation were harvested and then suspended in fresh medium (final cell density adjusted to OD_{750} 0.8). A disposable Petri dish (Iwaki; 8.5 mm diameter) containing 20 ml of the cell suspension was irradiated with a combination of cool-white fluorescent light ($500 \mu\text{W cm}^{-2}$) and UV-A light ($900 \mu\text{W cm}^{-2}$) from a UV-A lamp (Cosmo Bio; CSL-30A). Cells were harvested by centrifugation ($3500 g$ for 10 min at room temperature) at 100 min intervals over the course of 15 h, frozen using liquid nitrogen, and stored at -80°C until analysed.

Preparation of the water-soluble fraction from cyanobacterial cells and SDS-PAGE analysis. Pelleted cells stored at -80°C were thawed quickly, and suspended in disruption buffer containing 25 μl glycerol, 1 μl 10% SDS, 0.1 μl β -mercaptoethanol and 5 μl 200 mM phenylmethanesulfonyl fluoride, followed by the addition of 300 μl buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.5). The cells were ruptured by sonication for 15 min at 4°C , then centrifuged ($25000 g$, 30 min, 4°C).

The supernatant was denatured for 10 min at 100°C in 40 μl 0.0625 M Tris/HCl, pH 6.8, 1.25% SDS, 25% glycerol and 1.25% 2-mercaptoethanol. The denatured solution was centrifuged for 10 min and a sample of the supernatant (20 μl containing 100 ng protein) was analysed by SDS-PAGE on a 1.0 mm thick slab gel (10% acrylamide separating gel, 4% acrylamide stacking gel) using the buffer system of Laemmli (1970). The gel was stained with Coomassie brilliant blue. Protein concentration in the supernatant was determined by spectrophotometry at 205 nm (Scopes, 1974).

Protein purification and N-terminal amino acid sequencing. The target band in the stained gel was excised, and the protein was extracted by electroelution. The solution of the target protein was concentrated, and spotted on a PVDF membrane (Millipore; Immobilon-Psq). The N-terminal amino acid sequence was determined using a polypeptide sequencer (Shimadzu; PPSQ-10).

DNA sequencing of *groESL* and Northern analysis. Genomic DNA was extracted from cyanobacterial cells by standard procedures (Porter, 1988). Two primers for amplifying *groESL* from *Oscillatoria* sp. NKBG 091600 were designed based on conserved DNA regions in *groESL* sequences from *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 (ESF1, GAGTTCGGGAAMCCRYACT; ELR1, TCTTCGATYTG-GCGACGGATTG). The PCR product was cloned into pCRII vector (Invitrogen), and sequenced using an automatic DNA sequencing machine (Shimadzu; DSQ-1000L). A computer software package, LASERGENE (DNASTAR), was used for DNA sequence analysis.

Northern analysis was conducted using total RNA extracted from harvested cells according to the method of Chomczynski (1987). Digoxigenin (DIG)-labelled DNA probe for Northern hybridization was prepared by PCR amplification of a part of the *groEL* gene of *Oscillatoria* sp. NKBG 091600. Hybridization was performed using a DIG DNA Detection Kit (Boehringer Mannheim).

Primer extension analysis. Potential transcriptional start sites were identified by primer extension with RNA isolated from UV-A-irradiated cells. DNA-free total RNA (10 μg) and FITC-labelled primer (AGGGATACTGCTGCCATAGGAATAC-TCTCC: 40 pmol) were dissolved in 40 μl buffer [10 mM

Tris/HCl (pH 8.3), 50 mM KCl, 5 mM MgCl_2 , 1 mM each dNTP, 40 U human placenta ribonuclease inhibitor (Takara)] containing 10 U AMV (avian myeloblastosis virus) reverse transcriptase (Life Sciences). Extension reaction was carried out at 55°C for 1 h, following incubation at 99°C for 10 min to inactivate reverse transcriptase. The synthesized cDNA was ethanol-precipitated, washed with 70% ethanol and dissolved in 1 μl nuclease-free water. After adding 1 μl formamide loading dye (Thermo sequence fluorescent labelled primer cycle sequence kit; Amersham), the reaction mixture was heat-denatured at 95°C for 5 min and analysed by the DSQ-1000L automatic DNA sequencing machine in parallel with a dideoxynucleotide sequencing reaction primed with the same oligonucleotide.

RESULTS

Identification of a UV-A-inducible protein in *Oscillatoria* sp. NKBG 091600

Fig. 1 shows the SDS-PAGE pattern of water-soluble proteins extracted from the UV-A-resistant *Oscillatoria* sp. NKBG 091600 and the UV-A-sensitive *Synechococcus* sp. PCC 7942 (Hirose & Miyachi, 1983) after 15 h UV-A irradiation. Induction of a 60 kDa protein was observed in NKBG 091600. In contrast, induction of a corresponding SDS-PAGE band was not observed in the UV-A-sensitive PCC 7942.

Twenty-five N-terminal amino acid residues (without the first two amino acids) of the UV-A-responsive 60 kDa protein were determined. Homology searching using protein databases indicated that the 60 kDa UV-A-responsive protein sequence displayed similarity to cyanobacterial GroEL chaperonins (96% identity to *Synechococcus* sp. PCC 6301 and PCC 7942, and 84% to *Synechocystis* sp. PCC 6803). The 60 kDa protein cross-

Fig. 1. SDS-PAGE of water-soluble proteins extracted from *Synechococcus* sp. PCC 7942 (lanes 1 and 2) and *Oscillatoria* sp. NKBG 091600 (lanes 3 and 4) irradiated with UV-A. Lanes 1 and 3, cells were irradiated with a cool-white fluorescent light; lanes 2 and 4, cells were irradiated with a combination of cool-white fluorescent light and UV-A light for 15 h. M, size markers. The induced 60 kDa protein in lane 4 is indicated by an arrowhead.

The sequence of upstream region

Fig. 2. The *groESL* operon from *Oscillatoria* sp. NKBG 091600. Two putative -35 and -10 sequences, and the putative RBS sequence, within the upstream region of *groES* are underlined. The CIRCE and a putative SOS box are marked with bold underlining. The amino acid sequence with shaded background shows the deduced GroEL amino acid sequence matching that obtained from N-terminal sequencing of the 60 kDa protein.

reacted with antibody raised against GroEL of *Synechococcus* sp. PCC 7942 (data not shown).

Cloning and sequencing of the *groESL* operon of *Oscillatoria* sp. NKBG 091600

The *groESL* operon was amplified from genomic DNA of *Oscillatoria* sp. NKBG 091600 using PCR primers designed from conserved sequences in *groESL* of *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803. The DNA sequence of the amplified fragment (1.7 kbp) shows that the amplified fragment includes *groES* with 260 bp of upstream region and 1059 bp of the *groEL* 5' region (Fig. 2; GenBank AF054630). Part of the deduced GroEL amino acid sequence matched that obtained from N-terminal sequencing of the 60 kDa protein (Fig. 2, bottom). DNA sequence analysis revealed that *groES* and partial *groEL* of NKBG 091600 showed high similarity with the corresponding sequence from *Synechococcus* sp. PCC 7942 (74% identity at the nucleotide level). In contrast, the upstream region of

groES, and the region between *groES* and *groEL*, from NKBG 091600 exhibited poor similarity to corresponding sequences from *Synechococcus* sp. PCC 7942. A perfect inverted repeat (IR) of 9 bp (TTAGCACTC-N₉-GAGTGCTAA) is located 175 bp upstream of the *groES* translation initiation codon. This IR corresponds to the CIRCE (controlling inverted repeat of chaperone expression) element observed upstream of the *groESL* operon of *Bacillus subtilis* (Mogk *et al.*, 1997). The CIRCE in the other cyanobacterial strains *Synechococcus* PCC 7942 and *Synechocystis* PCC 6803, is located 55 bp and 57 bp upstream of the translation initiation codon, respectively, i.e. closer to the translation initiation codon than the CIRCE of NKBG 091600. Putative -35 and -10 hexamers were identified according to similarity with the -10 and -35 consensus region of the *groESL* operon of *Synechococcus* sp. PCC 7942 (-35 , GTTGCC; -10 , TACATT) (Webb *et al.*, 1990). The sequence CTGCTCAATCAACCAG (nt 141 to 156; the common motif is underlined), which resembles the SOS box consensus sequence of *Escher-*

in the UV-A-resistant *Oscillatoria* sp. NKBG 091600 but not in the UV-A sensitive *Synechococcus* sp. PCC 7942.

Two putative promoters and two operator sequences were found upstream of *groES* in NKBG 091600. A 9 bp IR corresponds to CIRCE elements, which have been shown to play an important role in the heat induction of the *groESL* operons of *B. subtilis* and other bacterial species including cyanobacteria (Hecker *et al.*, 1996; Mogk *et al.*, 1997). Compared to previously characterized CIRCEs, the CIRCE of NKBG 091600 is located further upstream of the predicted transcriptional start site of the *groESL* operon. A putative promoter overlapping the CIRCE motif was apparently not used during UV-A-induced transcription of the operon. However, a transcript starting from a promoter located more proximal to the translation start site was identified by primer extension analysis of mRNA extracted from UV-A-induced cells. The promoter of this transcript overlaps with an SOS consensus sequence. As near-UV light is known to generate O_2^- and H_2O_2 (Sammartano *et al.*, 1985; Shibata *et al.*, 1991; Tyrrell, 1985), it is plausible to assume that the SOS box is involved in the control of GroEL expression.

Hirosawa & Miyachi (1982) reported that cyanobacterial cells exposed to UV-A contained less chlorophyll and phycocyanin, and more carotenoid, than non-irradiated cells. We have also observed a decrease in the content of photosynthetic pigments, especially phycocyanin, in *Oscillatoria* sp. NKBG 091600 after UV-A irradiation; however, a recovery of phycocyanin content correlating with GroEL and biopterin glucoside induction was observed (data not shown). The temporal relationship of GroEL induction in NKBG 091600 to biopterin glucoside biosynthesis and the reconstitution of photosynthetic pigments suggests that this UV-A-inducible GroEL might function to upregulate biopterin glucoside biosynthesis, thereby allowing growth under UV-A irradiation.

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