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Research communication

Identification and expression of the gene product encoding a CPD photolyase from *Dunaliella salina*

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Abstract

Ultraviolet light induces photoproducts, cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs), in cellular DNA, which cause cytotoxic and genotoxic effects on the cells. Cells have several DNA repair mechanisms to repair the damage and to maintain genetic information of the cells. Photoreactivation is one of the DNA repair mechanism to remove UV-induced DNA damage from cellular DNA catalyzed by photolyase under visible light. Two types of photolyase, CPD photolyase and (6-4) photolyase, are specific for CPDs and for (6-4)PPs. We have isolated a gene product encoding CPD photolyase, named PHR2, from *Dunaliella salina* which is a kind of unicellular alga. Sequence analysis showed that PHR2 encodes a protein that has 529 amino acids and is similar to other Class II CPD photolyase. The complementation assay of the photoreactivation deficiency of the *Escherichia coli* SY2 by PHR2 cDNA showed a significant increase in survival rate when cells were irradiated with UV-C. Real-time PCR analysis indicated that the transcription of PHR2 was induced by UV-C, white light, high salinity, and H_2O_2.

Keywords: *Dunaliella salina*; PHR2; UV; Photolyase; Photoreactivation

1. Introduction

Solar UV radiation in the UV-B (315–280 nm) and UV-C (280–100 nm) spectral regions has mutagenic, carcinogenic, and lethal effects on living organisms [1–4]. The major damage induced in DNA by UV radiation is cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs) [5]. CPDs constitute the major class of these lesion (about 75%) and the rest mainly are (6-4)PPs [6]. Both classes of dimer may block transcription and DNA replication if left unrepaired [6,7]. Organisms have developed several paths, including nucleotide excision repair (NER) and photoreactivation, to repair DNA damage and keep the integrality of genetic information. Photoreactivation is a light-depend way to repair UV-induced DNA damage under the action of photolyase, i.e., CPD photolyase and (6-4) photolyase, CPD photolyase and (6-4) photolyase specifically binds to CPDs and (6-4)PPs, and repairs them, respectively [8].

All known photolyases contain a reduced FAD and a second chromophore which is either 5,10-methenyltetrahydrofolylpolyglutamate (MTHF) or 8-hydroxy-5-deazaflavin (8-HDF), depending on the source of the enzyme [5,9,10]. Then CPD photolyases are classified into two classes, I and II, based on their amino acid sequence similarity: Class I CPD photolyase genes have been isolated from prokaryotes and eukaryotes fungi, but all from unicellular organisms; Class II CPD photolyase genes have been isolated from a wide variety of organisms, including

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eubacteria, archaebacteria and higher eukaryotes [11,12]. The identity between Class I and Class II CPD photolyase is less than 20% [12].

CPD and (6-4) photolyase are both absent in placental mammals including human [8]. Many organisms posses CPD photolyase (including *Escherichia coli* and *Saccharomyces cerevisiae*), while some other organisms, including some plants, posses both types of photolyase [1]. However, both types of photolyase have been isolated from only a few organisms, including *Drosophila melanogaster* and *Arabidopsis thaliana* [13–16]. Two algal CPD photolyase genes have been isolated from the species *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa*. Different with other organismal CPD photolyase, *C. reinhardtii* CPD photolyase has carboxyl terminal tail of 90 amino acids. The function of this carboxyl domain is unknown. Deleting the region has no effect on the ability of photoreactivation [5].

Since our laboratory isolated the gene of (6-4) photolyase from *Dunaliella salina* in 2005 [13], we searched for the presence of another member of photolyase/blue-light photoreceptor. Now we have cloned and characterized the gene product of CPD photolyase from *D. salina*, named PHR2 and deduced that it belonged to Class II CPD photolyase through alignment with CPD photolyase from other organisms.

2. Materials and methods

2.1. Alga material and growth conditions

D. salina was grown in a controlled environment chamber with 16 h light and 8 h dark at 25 °C. The composition of the grown medium was 1.5 M NaCl, 5.0 mM $NaNO_3$, 5.0 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM $NaH_2PO_4 \cdot 2H_2O$, 1.0 mM KCl, 10.0 mM $NaHCO_3$, 0.3 mM $CaCl_2 \cdot 2H_2O$ and a mixture of micronutrients [17]. Cells in exponential phase were used for experiments.

2.2. Preparation of RNA

Total RNA was extracted with Trizol (Invitrogen) and separated on a MOPS-formaldehyde agarose gel to determine its quality (data not shown).

2.3. Degenerate oligonucleotides for amplification

Two degenerate PCR primers were designed based on the five reported Class II CPD photolyase amino acid sequences at two conserved sites shown in Fig. 1. The degeneracy of the primers was minimized by using inosines at most positions having fourfold redundancy that were close to the 3′ ends of the primers. The primers are:

Forward: 5′-GT(G,C,T,A)GA(T,C)GC(T,A)CA(T,C)A-ATGTIGTICC-3′

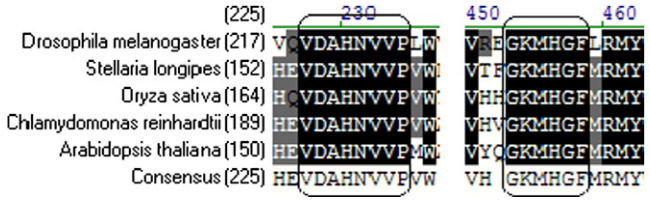

Fig. 1. Two conserved amino acid regions among CPD photolyases from five organisms. The sites used for primer design are circled.

Backward: 5′-(A,G)AA(G,C,T,A)CC(A,G)TGCAT(C,T)-TTICC-3′ (I = inosine)

2.4. PCR amplification of a CPD photolyase related cDNA fragment from D. salina

Total RNA was used for cDNA synthesis with the cDNA kit (TaKaRa RNA PCR Kit (AMV) Ver. 2.1) (TaKaRa) following the supplier's instruction. Using this cDNA as template and degenerate oligonucleotides as primers, a 708 bp CPD photolyase related cDNA fragment could be amplified.

2.5. Cloning of D. salina CPD photolyase

3′ RACE was performed using the 3′ RACE kit (3′-Full RACE Core Set) (TaKaRa). 5′ RACE was performed using the 5′ RACE kit (5′-Full RACE Core Set) (TaKaRa). Sequencing of the final clones was performed at Invitrogen.

2.6. Photolyase complementation assay

To identify the photoreactivating function of *D. salina* photolyase, a cDNA fragment of *D. salina* PHR2 encoding the photolyase was assembled in the vector pGEX-4T-1 (yielding pGEX-PHR2). Then the pGEX-PHR2 was transfected into *E. coli* strain SY2 (*uvrA*$^-$, *recA*$^-$, *phr*$^-$). SY2 was deficient in photoreactivation, nucleotide excision repair, and recombination, so it could be used as the host cell for photolyase complementation assay [18]. The transformants SY2/pGEX-PHR2 were grown in LB medium supplemented with 50 μg/ml ampicilin. The cultures were incubated at 37 °C until the OD_{600} reached 0.6, then isopropyl-β-D-thiogalactopyranoside (0.5 mM) was added and the cultures were incubated at 28 °C for 4 h. Appropriately diluted cells were spread on LB/Amp dishes and irradiated with 0.05, 0.15, and 0.25 J/m^2 UV-C light (Philips TUV TL-D, 15 W). One set sample dishes were exposed to fluorescent light from white lamps (Toshiba FL20S-D, 20 W) for 30 min before transfer to the dark, and the control set of dishes were transferred to the dark directly after irradiating with UV-C. The fluorescent light was filtered through a glass plate with a thickness of 2 mm to block out UV radiation present if any in the fluorescent light, and the minimum distance from the lamp to the cells was about 10 cm. The same experimental conditions were used with SY2/pGEX-4T-1 without PHR2 as the negative control. After incubation overnight at 37 °C surviving colonies were counted.