Journal of Invertebrate Pathology 105 (2010) 363–365

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

Amsacta moorei entomopoxvirus encodes a functional DNA photolyase (AMV025)

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ARTICLE INFO

Article history:
Received 3 March 2010
Accepted 25 June 2010
Available online 1 July 2010

Keywords:
Amsacta moorei entomopoxvirus
DNA photolyase
Chrysodeixis chalcites NPV
Photoreactivation
DNA repair

ABSTRACT

The major damage induced in DNA by ultraviolet light is the induction of cyclobutane pyrimidine dimers (CPDs). *Amsacta moorei* entomopoxvirus (AMEV) encodes a CPD photolyase (AMV025) with a putative role in converting these dimers back into monomers. In infected *Lymantria dispar* cells transcription of the AMV025 gene started 8 h post inoculation (p.i.) and continued through 38 h p.i. Transcription was inhibited by a DNA synthesis blocker. Transient expression in an *Escherichia coli* strain that lacks its endogenous photolyase, rescued growth of the UV-irradiated bacteria in a light-dependent manner, showing that AMV025 encodes a functional DNA photolyase.

1. Introduction

Exposure of DNA to far UV light (200–300 nm) produces various photoproducts (Sancar, 2003; Weber, 2005). Cyclobutane pyrimidine dimers (CPDs) are the most common and typically comprise 80–90% of the lesions formed. Photoreactivation is a light-dependent process mediated by photolyases, which utilize the energy of visible light to convert the dimers into monomeric pyrimidines (Hearst, 1995; Todo et al., 1993) and exist in all kind of organisms except placental mammals (Kato et al., 1994). To do so they use flavin adenine dinucleotide (FAD) as the catalytic co-factor as well as a light-harvesting co-factor (Deisenhofer, 2000; Sancar, 2003; Weber, 2005). Class II CPD photolyase (*phr*) genes (Kanai et al., 1997) are also found in members of the genera *Avipoxvirus* and *Leporipoxvirus* in the subfamily *Chordopoxvirinae* and in viruses in the subfamily *Entomopoxvirinae* (Afonso et al., 1999, 2000; Bawden et al., 2000; Cameron et al., 1999; Tulman et al., 2004; Willer et al., 1999). In chordopoxviruses these enzymes can repair UV damage in DNA (Bennett et al., 2003; Srinivasan et al., 2001; Srinivasan and Tripathy, 2005). In addition, photolyase genes are a typical feature for a sub-group of baculoviruses (Xu et al., 2008) and the *phr2* gene of *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV) encodes a functional CPD photolyase (van Oers et al., 2008). In this study we analyzed whether open reading frame (ORF) AMV025 (Bawden et al., 2000) of *Amsacta mori* entomopoxvirus (AMEV), the type species of the genus *Beta-entomopoxvirus*, encodes a biochemically active photolyase.

2. Materials and methods

Lymantria dispar Ld652 cells (Lynn et al., 1988) were infected at a m.o.i. of 2 pfu/cell with AMEV (supplied by Dr. R.W. Moyer, University of Florida, Gainesville, USA) and total RNA was isolated at various times post inoculation (p.i.). Aliquots of 10 µg were treated with 200 U of RNAse free DNAse I (Ambion) at 37 °C for 30 min and extracted with phenol–chloroform. Equal amounts of RNA were screened for the presence of AMV025-specific transcripts by RT-PCR. RNA isolated at 24 h p.i. from cells infected in the presence (or absence) of the DNA synthesis inhibitor Ara-C (Sigma; 100 µg/ml) was also analyzed. A reaction in which the RT step was omitted, confirmed the absence of DNA contamination.

The AMV025 ORF was amplified with flanking XmaI and HindIII sites from AMEV DNA and cloned in plasmid pKK223-3 (Amersham). The recombinant plasmid was used to transform bacteria of the *Escherichia coli* KY29 strain (JM107+*phr19*::Cm *recA56*) (Akasaka and Yamamoto, 1991). Fifty milliliters LB medium with ampicillin (200 mg/l) and chloramphenicol (34 mg/l) were inoculated with 100 µl overnight bacterial cultures containing pKK-AMV025, the empty plasmid pKK223-3, or a positive control plasmid expressing ChchNPV-*phr2* (van Oers et al., 2008). IPTG (40 mg/l) was added after incubation for 1.5 h at 37 °C and 3 h later bacteria were collected from 1 ml samples by centrifugation. Cell pellets were washed and suspended in 1 ml M9 salt solution (6.8 g/l Na_2HPO_4, 3 g/l KH_2PO_4, 0.5 g/l NaCl, 1 g/l NH_4Cl). Samples of 50 µl were diluted 100-fold and UV-irradiated at 254 nm with a dose of 0, 0.59, 1.14 or 2.85 J/m^2 in 3.5 cm glass petri dishes. Duplicate samples of 400 µl were collected in 24-well plates. One plate was illuminated for 30 min at 30 °C by Plexiglas-filtered white light provided by two Philips TL-D 36 W/840 fluorescent lamps at a

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doi:10.1016/j.jip.2010.06.013

distance of 30 cm, the other plate was kept in the dark. Suitably diluted samples were plated on LB-agar plates. The number of colonies was used to calculate the survival relative to the non-UV-irradiated controls. The results are based on two independent experiments.

3. Results and discussion

AMV025 encodes a protein of 453 amino acids. The region between amino acids 214 and 416 has homology to known FAD binding domains. A region characteristic of the N-terminus of DNA-photolyases and in general responsible for binding the light-harvesting chromophore (reviewed by Essen (2006)) was found by alignment with other CPD photolyases (Supplementary Fig. 1). AMV025 photolyase is not truncated compared to other photolyases and shows homology over its entire length. The putative AMEV photolyase shows amino acid sequence identities between 34% and 38% with the corresponding sequences from *Melanoplus sanguinipes* entomopoxvirus (MSEV) and several chordopoxviruses (Afonso et al., 1999, 2000; Cameron et al., 1999; Tulman et al., 2004; Willer et al., 1999). Slightly lower identities (26–33%) were observed with baculovirus photolyases (van Oers et al., 2004, 2005; Willis et al., 2005; Wang et al., 2007).

Phylogenetic analysis demonstrated that poxvirus photolyases are evolutionary separated from baculovirus photolyases (Supplementary Fig. 2). Avipox and leporipox *phr* genes were found on two separate branches in the phylogenetic tree and they appeared to have a common ancestor not shared with the entomopoxviruses lineage. The avipox *phr* gene lies in a region of conserved synteny with other chordopoxviruses (Gubser et al., 2004; Upton et al., 2003), suggesting common ancestry of their *phr* genes (Bratke and McLysaght, 2009). The *phr* gene in leporipoxviruses is not found at the same location, although synteny is conserved in this region for other genes, which may point to a separate *phr* insertion event. Gene order conservation is too limited between chordopoxvirus and entomopoxvirus genomes to draw conclusions on the origin of entomopoxvirus *phr* genes based on gene order.

To determine whether the AMV025 gene was expressed during AMEV infection total RNA isolated at various time points p.i. of Ld562 cells was analyzed. A single RT-PCR product was first detected at 8 h p.i. and reached a plateau at 14 h p.i. (Fig. 1A). Viral DNA replication is required for the expression of AMV025 since transcripts were not detected in the presence of a DNA synthesis inhibitor (Fig. 1B). Transcription in the late phase is in line with the timing of transcription of the Fowlpox virus (FPV) *phr* gene (Srinivasan et al., 2001). The region upstream of AMV025 contains two motifs (from position −57 to −38 and −21 to −2) that fit with the consensus vaccinia late promoter sequence TAAAT (Davison and Moss, 1989). Whether these late motifs are used for expression of AMV025 needs further analysis. Two motifs corresponding to vaccinia intermediate promoter motifs (TAAAAT) are also present upstream of AMV025, but are apparently not used, since no AMV025 transcripts were observed when the infection was halted in the intermediate stage (Fig. 1B). The timing of transcription may be different than for baculovirus *phr* genes, which are probably expressed as early genes (van Oers et al., 2004, 2005; Willis et al., 2005).

To examine whether AMV025 encodes a functional photolyase it was expressed in photolyase deficient *E. coli* cells. A clear difference was observed in survival between samples exposed to visible light and those kept in the dark following UV treatment as expected for a functional DNA photolyase (Fig. 2). For instance at a dose of 0.59 J/m^2 bacteria transformed with the plasmid carrying AMV025 showed a survival of 60% when treated with visible light compared to 11.5% in the dark. The survival with the empty vector was below 5% in all cases and did not show a difference between light and dark treatment.

An important factor determining whether a poxvirus could benefit from a *phr* gene, may be whether the virus has to survive outside the host in the presence of UV light or whether it is frequently transmitted by direct contact between susceptible hosts, as in the case of small pox- and monkeypoxvirus, which lack *phr* genes. The presence of a *phr* gene makes the fowl pox virus less UV sensitive (Srinivasan and Tripathy, 2005) and allows the virus to survive for longer periods of time in the absence of a suitable host. The presence of occlusion bodies (spheroids), which surround the entomopoxvirus particles (Arif, 1996) and presumably protect the viruses against environmental influences, similar to baculovirus occlusion bodies, suggest that entomopoxviruses, like baculoviruses, have an ecology with an extended period outside the host. As the entomopox photolyase is encoded by a late gene, it may end up in the virus particle as does the FPV photolyase (Srinivasan and Tripathy, 2005). Recent LC–MS/MS studies have revealed that the AMEV

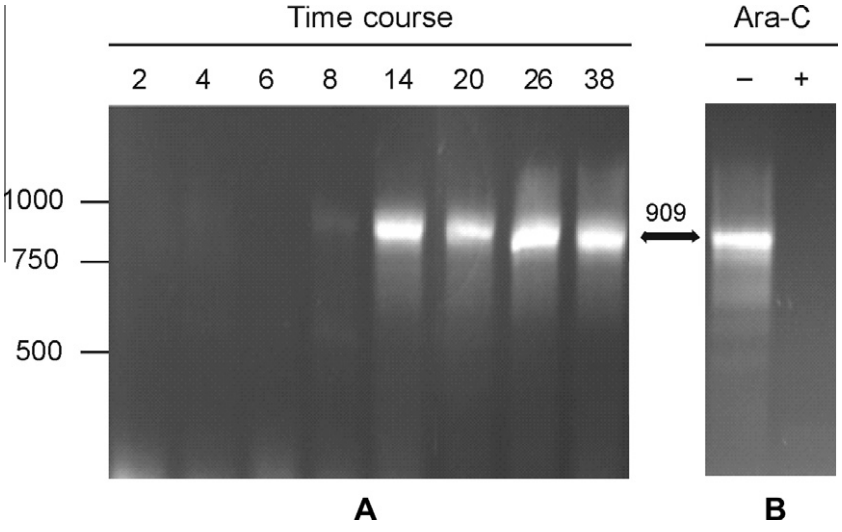

Fig. 1. Transcriptional analysis of the AMV025 gene. (A) Time course of AMV025 gene transcripts detected by RT-PCR analysis. The time points post inoculation when RNA samples were extracted from AMEV-infected Ld652 cells are indicated in hours. (B) The temporal class of the AMV025 gene was determined by RT-PCR analysis in the absence (−) or presence (+) of the DNA synthesis inhibitor Ara-C. The amplicons were analyzed in 1.2% agarose. The amplicon size is indicated between the gels and the marker sizes on the left (in bp).

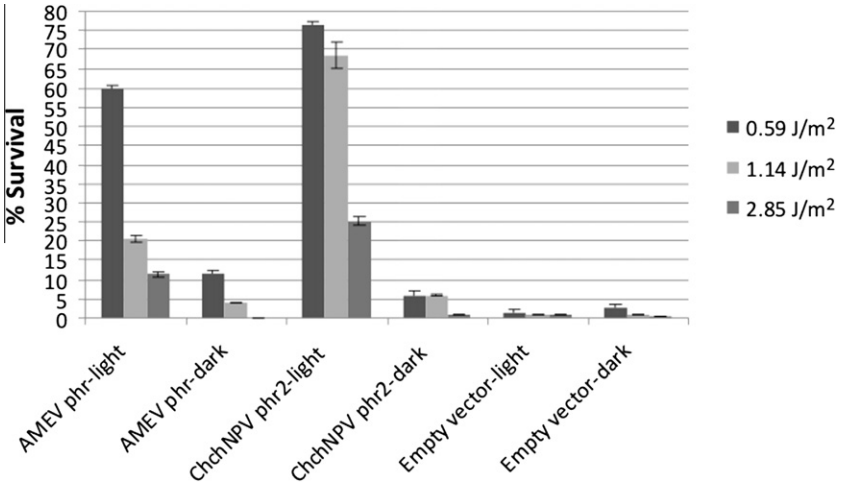

Fig. 2. Photoreactivation of UV-induced damage in repair-deficient *E. coli* KY29 cells harboring plasmids pKK-AMV025, pKK-Ccphr2 or the empty pKK233–3 vector. Cells were treated at three different UV doses. After UV irradiation, cells were kept in the dark or illuminated with white light for 30 min. Percentage survival is given in relation to the survival of non-UV-irradiated cells containing the same plasmid. Error bars represent the standard deviation of the mean based on two independent experiments.

photolyase is indeed associated with the occluded virion (Perera and Arif, personal communications).

In conclusion, AMV025 is a late gene and encodes a functional photolyase, which can catalyze UV damage repair in a light-dependent manner and may protect the virus against UV irradiation.

Acknowledgments

We are grateful to Basil Arif for his scientific support and for sharing highly relevant data prior to publication. This research was supported by a grant (BIDEB) from the Scientific and Technological Research Council of Turkey to Remziye Nalcacıoğlu. Monique van Oers was sponsored by a MEERVOUD grant from the Netherlands Organisation for Scientific Research (NWO).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jip.2010.06.013.

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