Journal of Photochemistry and Photobiology B: Biology 116 (2012) 95–104

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Protective effect of low UVA irradiation against the action of lethal UVA on *Pseudomonas aeruginosa*: Role of the *relA* gene

Magdalena Pezzoni, Ramón A. Pizarro, Cristina S. Costa *

Comisión Nacional de Energía Atómica, Departamento de Radiobiología, Argentina

ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form 17 July 2012
Accepted 9 August 2012
Available online 21 August 2012

Keywords:
Pseudomonas
Ultraviolet-A (UVA)
relA
Stringent response
Osmotic stress

ABSTRACT

The exposure of *Pseudomonas aeruginosa* cells to very low UVA fluences induces a growth delay, a phenomenon proposed in *Escherichia coli* as an adaptive mechanism related to protection against lethal and mutagenic effects of UVA. This paper reports that the treatment with low UVA irradiation fluences protects *P. aeruginosa* PAO1 strain from a subsequent lethal exposure. This phenomenon depends on the *relA* gene, coding for the main (p)ppGpp synthetase, and is unrelated to the induction of quorum sensing or catalase activity, two essential factors involved in the response of *P. aeruginosa* to UVA. Cross-protection between osmotic stress and UVA is observed when a great protective response to lethal UVA is caused by the induction of resistance to osmotic stress. The increase in resistance to osmotic shock observed in the pre-irradiated PAO1 strain but not in its *relA* derivative, unable to show photo-protection, leads us to hypothesize that the photo-protection could be attributed to an adaptive response to osmotic stress. It is concluded that the exposure of *P. aeruginosa* to low UVA doses induces a *relA*-dependent adaptive response that protects against cell death induced by high doses and causes an increase in the resistance to osmotic stress.

1. Introduction

One of the most stressing agents for bacteria present in aquatic or terrestrial environments is solar ultraviolet-A (UVA) radiation (400–315 nm), the major fraction of ultraviolet radiation reaching the Earth's surface. A review focusing on the effects of UVA on survival and growth of Gram negative bacteria has been recently published [1]. It is widely accepted that high doses of UVA lead to lethal effects mainly via reactive oxygen species, which produce oxidative damage to proteins and lipids, and finally cause cell inactivation [2,3]. On the other hand, low UVA doses induce a transient inhibition of growth called growth delay [4]. This sublethal effect was investigated by several researchers and the phenomenon was proposed as an adaptive mechanism against the lethal and mutagenic effects of higher doses of UVA and solar irradiation [5].

In *Escherichia coli*, the growth delay phenomenon was ascribed to a restriction in the aminoacylation capacity of certain tRNAs by the formation of a cross-link between 4-thiouridine in position 8 and cytidine in position 13, attributed to the UVA absorption by the thiolated nucleoside [6,7]. The presence of uncharged tRNAs due to UVA action leads to a *relA*-dependent increase in the level of two guanosine nucleotides, ppGpp and pppGpp, hereinafter called (p)ppGpp, resembling the mechanism known as stringent response [8]. In addition to the stringent response, oxidative disturbance of bacterial membranes induced by sublethal UVA exposure contributes to increasing the UVA-dependent growth delay [9].

The stringent response is an adaptive mechanism that allows the bacteria to adapt rapidly to different environmental stress conditions, such as nutritional starvation. In this phenomenon, the accumulation of (p)ppGpp represses the transcription of genes involved in RNA and protein biosynthesis with the consequent arrest of bacterial growth, and the induction of genes involved in protective functions is promoted [10]. RelA enzyme is the main synthetase of (p)ppGpp, and is activated in response to amino acid deprivation [11]. The regulatory mechanism of the stringent response also involves the bifunctional enzyme SpoT, which hydrolyses the guanosine nucleotides and has a minor (p)ppGpp synthetase activity. Accumulation of (p)ppGpp by SpoT depends on other stress factors different from amino acid deprivation, such as carbon depletion, membrane perturbations and inhibition of fatty acid metabolism [12–14].

The growth delay phenomenon caused by exposure to low UVA doses was recently described in *Pseudomonas aeruginosa* [15]. In this study, it was demonstrated that the quorum sensing system rhl is involved in this response mainly for its role in antioxidative

* Corresponding author. Address: Comisión Nacional de Energía Atómica, Departamento de Radiobiología, Avda. General Paz 1499, B1650KNA General San Martín, Buenos Aires, Argentina. Tel.: +54 11 6772 7011; fax: +54 11 6772 7188.
E-mail addresses: pezzoni@cnea.gov.ar (M. Pezzoni), pizarro@cnea.gov.ar (R.A. Pizarro), costa@cnea.gov.ar (C.S. Costa).

1011-1344/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jphotobiol.2012.08.011

defense. The nucleotide 4-thiouridine, the UVA target related to growth delay, was described in *P. aeruginosa* long ago [16] and its relative proportion in the tRNA was found to be 13% less than in *E. coli* [17]. Later, Fernández and Pizarro reported that the accumulation of (p)ppGpp in *P. aeruginosa* is a consequence of UVA irradiation [18].

Bacterial adaptive responses usually include changes in the expression of genes related to stress tolerance in response to environmental stimuli. Sublethal exposure to diverse stress factors can usually increase survival after subsequent environmental challenges. To the best of our knowledge, the only experimental evidence of adaptive response generated by low doses of UVA irradiation to a subsequent lethal exposure of the same stress agent has been reported in *E. coli* [19], demonstrating that the exposure to continuous sublethal UVA irradiation leads to an increase in survival after subsequent lethal UVA irradiation, a change in the activity levels of antioxidant enzymes and a reduction in the duration of growth delay.

This study reports the protective action of pre-exposure to low UVA fluences capable of reducing the lethality induced by subsequent high dose exposure in *P. aeruginosa*. Mechanisms involved in this phenomenon were investigated and cross-protection against osmotic challenge is reported.

2. Materials and methods

2.1. Bacterial strains, plasmids and culture conditions

Table 1 lists the strains and plasmids used in this study. Plasmids were maintained with 100 μg ml^{-1} ampicillin in *E. coli* or 200 μg ml^{-1} carbenicillin in *P. aeruginosa*.

Bacterial cultures were grown at 37 °C with shaking in complete Luria–Bertani broth [20]; for solid medium, 15 g l^{-1} agar was added. This medium, hereafter called LB, contained 0.085 M NaCl; different final NaCl concentrations were used when necessary.

2.2. Irradiation source

A bench with two Philips TDL 18W/08 tubes was used. According to the information provided by the manufacturer, more than 95% of the emission from the tubes is at 365 nm. The incident fluence rate in our experimental conditions was measured at the surface of the suspensions with a 9811.58 Cole-Parmer Radiometer (Cole-Parmer Instruments Co., Chicago, IL, USA). The UVA tubes were mounted on aluminum anodized reflectors enhancing the fluence rate on the section to be irradiated. The fluence rate, or the energy per unit area per unit time, was expressed in W m^{-2}; the total dose, which was calculated based on the fluence rate and the exposure time, was expressed in kJ m^{-2}.

To evaluate the possible participation of wavelengths below the UVA band, which, even in a very low proportion, could affect cell viability, some experiments were repeated with irradiation through glass. The response of the strains thus irradiated was the same as that of strains for which the open surface was irradiated, indicating that the results are specifically due to the UVA radiation.

2.3. Standard irradiation procedure

Bacterial cultures were washed once and suspended in saline solution (NaCl 0.1 M) at an OD_{650} of 0.4. The suspension was divided into two 50 ml fractions, which were each placed in a glass beaker (diameter of the exposed surface 10 cm) open to air. One of these fractions was irradiated from above at a fluence rate of 28 W m^{-2} at the level of the free surface of the suspension while the other fraction remained in the dark. Both suspensions were kept in an ice bath under slow magnetic stirring throughout the procedure. Samples of both fractions were taken in order to measure cell viability.

2.4. Pre-treatment with sublethal UVA irradiation and subsequent exposure to lethal UVA fluences

The procedure is based on a previous paper [21], with modifications. Cultures at stationary growth phase were washed once and suspended in saline solution (NaCl 0.1 M) at an OD_{650} of 0.4. The suspension was divided into two 50 ml fractions, each of which was placed in a glass beaker (diameter of the exposed surface 10 cm) open to air. One of these fractions was irradiated from above at a fluence rate of 28 W m^{-2} for 20 min (total dose 34 kJ m^{-2}) at the level of the free surface of the suspension while the other fraction remained in the dark as control. Both suspensions were kept in an ice bath under slow magnetic stirring during the procedure. After irradiation, the suspensions were centrifuged and suspended in pre-warmed LB medium at an OD_{650} of 0.1. Control and pre-irradiated cells were then incubated in a bath at 37 °C with shaking until an OD_{650} of 0.3, harvested by centrifugation and suspended again in saline solution at an OD_{650} of 0.4. These suspensions were divided into two fractions: one fraction was irradiated at 28 W m^{-2} for 180 min (total dose 302 kJ m^{-2}) under the conditions described above and the other fraction was kept in the dark as control. Samples of each of the four fractions were taken in order to measure cell viability.

2.5. Survival curves and growth delay measurements

In order to measure cell viability, samples of the suspensions were taken during irradiation at the times indicated and plated on LB solid medium after dilution. Plates were incubated in the

Table 1
Strains and plasmids used in this study.

Strain or plasmid	Relevant genotype and/or phenotype	Source or reference
P. aeruginosa		
PAO1	Wild-type	B.W. Holloway
PAO-JP2	*lasI*::Tet *rhlI*::Tn*501-2*	[50]
PW2696	*relA*::Is*lacZ*/hah	Washington Genome Center [51]
PW8190	*katA*::Is*lacZ*/hah	Washington Genome Center [51]
PW8769	*katB*::Is*lacZ*/hah	Washington Genome Center [51]
E. coli		
DH5α	F⁻ Φ80d*lacZΔM15* Δ(*lacZYA-argF*)U169 *endA1 recA1 hsdR17 deoR gyrA96 thi-1 relA1 supE44*	Gibco
Plasmids		
pKDT1.7	*lasB′–lacZ lasR* (reporter of 3OC12-HSL)	[50]
pECP61.5	*rhlA′–lacZ ptac-rhlR* (reporter of C4-HSL)	[50]

All the *P. aeruginosa* strains are derivatives of PAO1 strain.