Environmental and Experimental Botany 74 (2011) 280–288

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Ultraviolet-B-induced DNA damage and photorepair in the cyanobacterium *Anabaena variabilis* PCC 7937

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ARTICLE INFO

Article history:
Received 9 October 2010
Received in revised form 4 June 2011
Accepted 24 June 2011

Keywords:
Cyanobacteria
DNA damage
DNA strand breaks
Photoreactivation
Thymine dimers
Ultraviolet radiation

ABSTRACT

The impact of simulated solar radiation on DNA and the mitigation of DNA-damaging effects by photoreactivation was studied in a cyanobacterium *Anabaena variabilis* PCC 7937. Cultures were irradiated under 295, 320 and 395 nm cut-off filters as well as seven other filters such as WG 280, WG 295, WG 305, WG 320, WG 335, WG 345 and GG 400. Growth of the test organism was found to be affected mostly under UV-B radiation as compared to PAR and PAR + UV-A radiations. Amplification of 16s rDNA and RAPD profile was significantly affected following exposure of genomic DNA to UV-B radiation. The formation of T<>T CPDs was recorded only in the cultures irradiated with UV-B radiation (i.e., under 295 nm as well as under WG 280, WG 295 and WG 305 nm cut-off filters), but maximum yield was found under 280 nm cut-off filter. Furthermore, the considerable induction of thymine dimers was observed with increasing UV-irradiation times. Fluorometric analysis of DNA unwinding (FADU) assay for UV-induced DNA strand breaks exhibited the maximum loss in the percentage of dsDNA under UV-B radiation followed by UV-A and PAR in comparison to the light control samples. We observed that T<>T CPD repair is light-dependent, since these lesions were more efficiently removed upon exposure to visible light than in the darkness. Blue radiation was found to be the most effective in photoreactivation than any other wavebands of light. Furthermore, the rate of photoreactivation was measured under varying temperatures (10, 20 and 30 °C); the repair rate was found to be the maximum at 20 °C under white fluorescent light. Our results indicate that photoreactivation play an important role in survival of the organism under natural conditions in spite of being exposed to the UV-B component present in the solar drops.

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1. Introduction

Cyanobacteria are one of the most dominant groups of photolysis mediated oxygen evolving, photoautotrophic, Gram-negative organisms having cosmopolitan distribution in both aquatic as well as terrestrial ecosystems ranging from hot springs to the Arctic and Antarctic regions. Cyanobacteria also occupy an important place in other extreme environments such as high salinities, pH and irradiances as well as in the form of endosymbionts in plants, lichens and several protists (Stanier and Cohen-Bazire, 1977; Carr and Whitton, 1982; Castenholz, 1996; Thajuddin and Subramanian, 2005; Taton et al., 2006; Dorador et al., 2008). They appeared and flourished on the Earth during the Precambrian era (2.8 and 3.5×10^9 years ago) with the creation of oxygenic environment and provided a favorable condition for the evolution of existing aerobic life (Brocks et al., 1999; Sergeev et al., 2002; Olson, 2006; Fischer, 2008). The most common cyanobacterial structures in the fossil record include stromatolites and oncolites (Herrero and Flores, 2008). It is believed that the credit for present eukaryotic plant life over the planet goes to cyanobacteria, as the chloroplast of eukaryotic cell is supposed to be of cyanobacterial origin (Tomitani et al., 2006).

Cyanobacteria are most important biomass producers and play globally significant role in biogeochemical cycle of nitrogen, carbon and oxygen (Häder et al., 2007). Inherent capabilities of cyanobacteria to fix atmospheric nitrogen by means of an enzyme nitrogenase makes them ecologically important source of natural biofertilizers for rice-growing countries and other N_2-deficient habitats (Vaishampayan et al., 2001). These prokaryotes are excellent source of a wide range of biologically active compounds of significant value that has fascinated the researchers for their pharmaceutical and biotechnological exploitations (Gademann and Portmann, 2008; Rastogi and Sinha, 2009; Rastogi et al., 2010a). Several species of cyanobacteria are known to produce hydrogen as a renewable source of energy. Production of molecular hydrogen by cyanobacteria may be a striking substitute over other conventional hydrogen

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doi:10.1016/j.envexpbot.2011.06.010

production processes, as it is an ecofriendly, proficient, renewable and commercially feasible alternative of energy (Dutta et al., 2005). Cyanobacteria can be transformed by gene alteration to produce ethanol with high efficiency (Deng and Coleman, 1999). Since long cyanobacteria are also being used as a food supplement (Gantar and Svircew, 2008).

In the past few decades, rapid progress in urbanization and industrialization has resulted in an increase in anthropogenically released chlorofluorocarbons (CFCs), chlorocarbons (CCs) and organobromides (OBs) causing depletion of the stratospheric ozone layer followed by increase in ultraviolet (UV) radiation (280–400 nm) on the Earth's surface (Crutzen, 1992; Lubin and Jensen, 1995; McKenzie et al., 2007). The incidence has generated tremendous concern about the negative impact of highly energetic solar UV-B (280–315 nm) radiation on aquatic as well as terrestrial life forms (Pfeifer et al., 2005; Häder et al., 2007; Norval et al., 2007; Lesser, 2008; Ma and Gao, 2009). Cyanobacteria depend on solar energy for their certain important energy-dependent processes such as photosynthesis and nitrogen fixation. Harvesting of solar radiation exposes them simultaneously to harmful doses of UV-B and UV-A (315–400 nm) radiations in their natural habitats and can affect a number of physiological and biochemical processes such as growth, survival, cell differentiation, pigmentation, heterocyst differentiation, motility and orientation, photosynthesis, enzymes of nitrogen metabolism, genome integrity and total protein profiles (Sinha et al., 1995, 2008a, 2008b; Gao et al., 2007, 2008; Lesser, 2008; Ma and Gao, 2009).

One of the most prominent targets of solar UV-radiation is cellular DNA, which absorbs UV-B radiation and attributes adverse effects on living systems (Sinha and Häder, 2002a; Häder and Sinha, 2005). Although UV-B has less than 1% of total energy, it is a highly active component of the solar radiation that directly brings about chemical modifications in DNA, and changes its molecular structure by the formation of dimers or indirectly affects them via the production of reactive oxygen species (ROS) (Vincent and Neale, 2000; Halliwell and Gutteridge, 2007). UV radiation-induced production of ROS in cyanobacteria was reported by He and Häder (2002a) using a ROS-sensitive probe, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). More recently, Rastogi et al. (2010b) detected the simulated solar UV radiation-induced generation of intracellular ROS in a cyanobacterium by direct fluorescence microscopic image analysis using DCFH-DA. Cis-syn cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4 PPs) and their Dewar isomers are the main DNA lesions produced by UV-B radiation that can alter the molecular structure of genome leading to chronic mutagenesis and death of the cell (Sinha and Häder, 2002a; Friedberg et al., 2006; Rastogi et al., 2009). Contrary to this, oxidative stress (by means of ROS) usually results in single- as well as double-strand breaks (DSBs) in the native DNA molecule (He and Häder, 2002b). In comparison to UV-B, the wavelength of UV-A has poor efficiency in inducing the DNA damage because they are not absorbed by native DNA. However, UV-A or visible light photon (up to 670–700 nm) are still able to induce DNA damage either by producing a secondary photoreaction of existing DNA photoproducts or via indirect photosensitization reactions (Hargreaves et al., 2007; Jiang et al., 2009). In all the groups of UVR, UV-C is quantitatively absorbed by oxygen and ozone in the Earth's atmosphere, hence, dose not show much harmful effects on biota.

Overall, DNA lesions constitute ubiquitous threat to the accurate maintenance of the genome, and their incidence and importance have motivated the development of precise cellular repair functions, some of which have been highly conserved during evolution. Although repair mechanisms of photoproducts have generally focused on plants, bacterial, yeast and mammalian systems (Britt, 1996; Dany et al., 2001; Hays, 2002; Bray and West, 2005; Kunz et al., 2005; Friedberg et al., 2006; Roldán-Arjona and Ariza, 2009), very few informations are available on DNA repair processes in cyanobacteria (Levine and Thiel, 1987; Mühlenhoff, 2000; Ng and Pakrasi, 2001). In the present investigation an attempt has been made to elucidate the impacts of UV radiation on the formation of thymine dimers as well as DNA strand breaks in the cyanobacterium *Anabaena variabilis* PCC 7937. In addition, photoreactivation of damaged DNA under various radiation regimes and temperatures have been studied.

2. Materials and methods

2.1. Strain and growth conditions

The cyanobacterium *A. variabilis* PCC 7937 was obtained from Pasteur culture collection (Institute Pasteur, France) and were routinely grown under axenic conditions in an autoclaved BGA^- (without nitrogen) liquid medium (Safferman and Morris, 1964) in a culture room at a temperature of $20 \pm 2\,°C$ and continuous fluorescent white light of $12 \pm 2\,W\,m^{-2}$. All the experiments were performed with exponentially growing cultures.

2.2. Source and mode of UV-irradiation

Cultures were exposed to a solar simulator (1000 W, Dr. Hönle GmbH, Martinsried, Germany) in open glass Petri dishes (75 mm in diameter) or in black boxes (5 cm × 5 cm). Three cut-off filters such as 395 filter foil (Ultraphan, UV Opak Digefra, Munich, Germany), 320 (Folex PR Montagefolie 320 nm Art. Nr. 10155099, Folex, Dreieich, Germany) and 295 nm (Ultraphan, Digefra) were used to obtain the desired radiation regimes of PAR, PAR + UV-A, and PAR + UV-A + UV-B respectively. The spectral irradiances of the solar simulator as transmitted by all three cut-off filters have been published earlier (Rastogi et al., 2010b). Besides the above-mentioned cut-off filters some other filters such as WG 280, WG 295, WG 305, WG 320, WG 335, WG 345 and GG 400 (Schott and Gen. Mainz, Germany) were also used to achieve the desired radiations. The irradiances effectively received by the samples through the filters of WG and GG series were similar as described by Gröniger and Häder (2002). The irradiance of UV was measured with a double-monochromator spectroradiometer (OL 754, Optronic Laboratories, Orlando, FL, USA).

2.3. Growth determination

Growth was measured at desired time intervals by estimating protein content by using the method as described by Bradford (1976). Bovine serum albumin was used as protein standard. Growth of *A. variabilis* PCC 7937 under simulated solar radiation with 395 (PAR), 320 (PAR + UV-A) and 295 (PAR + UV-A + UV-B) nm cut-off filters were measured up to 72 h while for the cut-off filters from WG 280 to GG 400, growth was measured after 48 h of irradiation under solar simulator.

2.4. Isolation of DNA and spectroscopic analysis

Isolation of genomic DNA was done as per the method described by Sinha et al. (2001). Exponentially growing cyanobacterial cultures were centrifuged at 4000 × *g* for 3 min (Centrifuge 5702, Eppendorf, Hamburg, Germany) and the pellet was transferred to a 1.5 ml nuclease-free microcentrifuge tube. The pellet was washed twice with TE buffer. Thereafter, 400 μl lysis buffer (Urea 4 M; Tris–HCl 0.2 M, pH 7.4; NaCl 20 mM and EDTA 0.2 M) and 50 μl proteinase k (20 mg/ml) was added and mixed well. This mixture was incubated for 1 h at 55 °C. Thereafter, 1 ml of extraction buffer (CTAB 3%; NaCl 1.4 M; EDTA 20 mM; Tris–HCl 0.1 M, pH

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