DETECTION OF UV-B-INDUCED THYMINE DIMER IN A CYANOBACTERIUM, SCYTONEMA SP.

Pavan Kumar

Address(es): Pavan Kumar, Department of Remote Sensing, Faculty of Earth Science, Banasthali University, Tonk, Rajasthan-304022, India.

*Corresponding author: pawan2607@gmx.com

ARTICLE INFO

Received 24. 5. 2013
Revised 29. 11. 2013
Accepted 2. 12. 2013
Published 1. 2. 2014

Regular article

ABSTRACT

DNA molecule is one of the major targets for UVR that can alter its molecular structure by forming different types of lesions leading to chronic mutagenic and even death of the cell. 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. Before assessing the impact of UV-B radiation on DNA, we observed its effects on growth and survival of the test organism Scyttonema sp. It was observed that growth and survival were severely affected by UV-B radiation for different durations. UV-B treatment causes loss in the cooperative binding property of DNA which is evident from the failure of complementary strands of DNA.

INTRODUCTION

INTRODUCTION

Ultrapurple is arbitrarily subdivided into three wavebands such as UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (<280 nm) that may affect the normal life processes of all organisms ranging from prokaryotes to mammals (Friedberg et al., 2005). In all the groups of UVR, UV-C is quantitatively absorbed by oxygen and ozone in the Earth’s atmosphere, hence, does not show much harmful effects on biota, but on the other hand UV-B and UV-A radiation play a significant role in producing the pronounced effects on diverse habitats, even though most of the extraterrestrial UV-B is absorbed by the stratospheric ozone (Madronich et al., 1998; McKenzie et al., 2003). UVR can affect a number of physiological and biochemical processes such as growth and survival, pigmentation, heterocyst differentiation, photosynthesis, enzymes of nitrogen metabolism and total protein profiles (Sinha et al., 1995; Sinha et al., 1998) in diverse organisms. DNA molecule is one of the major targets for UVR that can alter its molecular structure by forming different types of lesions leading to chronic mutagenic and even death of the cell. 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 is able to generate singlet oxygen (O2), or reactive oxygen species (ROS) that can damage DNA via indirect photosensitization reactions (Absher et al., 1977; Mackerness, 1999).

Impacts of UV radiation on Cyanobacteria

Cyanobacteria are phylogenetically a group of gram-negative prokaryotes that possess higher plant type oxygenic photosynthesis. During Precambrian period (between 2.8 to 3.5 x 10^9 years) they were probably the main primary producers to release oxygen into the then oxygen-free atmosphere. Hence, cyanobacteria seem to be leading organisms for the development of aerobic metabolism and rise of higher plants (Schoepf, 1993; Pace, 1997).

Cyanobacteria depend on solar radiation as their primary source of energy in their natural environment. The potential threat to these organisms is the continuous depletion of the stratospheric ozone layer and the consequent increase in solar UV-B radiation reaching the Earth’s surface (Blumthaler et al., 1990; Crutzen, 1992). Biologically effective doses of UV-B radiation may affect a number of physiological and biochemical processes of cyanobacteria such as growth and survival, pigmentation, total protein profiles, photosynthetic enzymes, photosynthesis, etc. UV-B mediated effects on growth and survival have been observed in several cyanobacteria (Tyagi et al., 1992; Sinha et al., 1995; Sinha et al., 1996; Quesada and Vincent, 1997; Prasad and Zeeshan, 2004). Effects of UV-B on growth and survival depends upon the species type, however, it has been shown that exposure of UVR for 120-180 min can completely cease growth and survival of several cyanobacteria (Tyagi et al., 1992; Sinha et al., 1995). Cyanobacterial strain such as Nostoc commune and Scyttonema sp. whose filaments are embedded in a mucilage sheath are more tolerant to UV-B radiation than the species which do not have such coverings (Sinha et al., 1996). Two Antarctica cyanobacteria such as Phoridium murrayi and Oscillatoria priestleyi, isolated from a common habitat show differential sensitivity to UV-B radiation.

UV-B induced DNA damage in cyanobacteria

It is apparent that DNA is the most prominent targets of solar UVR in all living organisms continuously incur a myriad of types of damage that drastically alter the normal life processes of all living systems such as bacteria, cyanobacteria, phytoplankton, macroalgae, plant, animals and humans (Peak and Peak, 1984; Quirke et al., 1992; Kripke et al., 1992; Pakker et al., 2000; Sinha et al., 2001; Buma et al., 2001; Jans et al., 2005) and influence their normal life processes. DNA alteration may occur mainly by mispairing of bases during replication, hydrolytic deamination, depurination/depyrimidination, oxidative damage by ionizing radiation (IR) as well as by free radicals or reactive oxygen species (ROS) and by certain alkylating agents (Dizdaroglu, 1992; Lindahl, 1993; Hallwell et al., 1999; Valko et al., 2006). The incidence of UVR, IR and certain genotoxic chemicals may result in single as well as double DNA strand breaks (DSBs). DSBs may lead to loss of genetic information. The high concentration of ROS may alter the configuration of cell structure, lipids, proteins and DNA and can cause a number of human diseases (Valko et al., 2007). Certain antibiotics (eg. mitomycin-c, cisplatin, etc.) may cause cross-linking by the formation of covalent bonds between two bases on complementary DNA strands and interfere with DNA replication. The most potent carcinogenic forms of UV-induced DNA lesions are CPDs, 6-4PPs and their Dewar isomers (You et al., 2000; Cadet et al., 2005) that may impede with normal cellular capability and functional integrity, reduction of RNA synthesis, arrest of cell cycle progression, resulting in mutagenesis, tumorigenesis and apoptosis (Van Steeg and Kraemer, 1999; Jans et al., 2005). It has been found that thymine-thymine (T-T) and thymine-cytosine (T-C) sequences are more photo-reactive than C-T and C-C sequences (Courdavault et al., 2005). The elevated amount of CPDs are produced by cycloaddition reaction between two pyrimidine bases in single-strand DNA (ssDNA) as well as at the flexible ends of poly (dA) (dT) tracts, but not at their rigid centre (Carell and Apple, 1998; Becker and Wang, 1989; Lymachiev, 1991). The yield ratio of CPDs and 6-4PPs mostly depends upon the two adjacent bases involved in the formation of dimer, though, it has been reported that the amount of CPDs and 6-4PPs are about 75 and 25 % respectively of the total UV-induced DNA damage product (Sinha and Hader, 2002). It is remarkable, and
very significant in terms of UV-induced toxicity, that RNA polymerase has also been found to stall at both CPDs and 6-4PPs (Britt, 1999). UV-induced formation of thymine dimers have been reported in a number of rice field cyanobacteria such as Anaabaena sp., Nostoc sp. and Scytonema sp. (Sinha et al., 2001). UV-induced DNA degradation has been demonstrated in the unicellular cyanobacterium Synechocystis PCC 6308. Recently, UV-B-induced damage of DNA and its detection by PCR have been reported in cyanobacteria (Kumar et al., 2004). It seems probable that UV affects the DNA of cyanobacteria and the killing of these microbes might be due to the irreversible damages caused to DNA by the high energy UVR (Kumar et al., 2004). UV-B-induced formation of thymine dimer has also been reported in cyanobacteria, such as Croococcus sp. and Anaabaenaopsis sp.

Detection of DNA damage

A immuno-dot-blot assay was used to detect CPDs, 6-4PPs and their Dewar valence isomers in UV-irradiated mammalian organisms (Perdiz et al., 2000). To determine the frequency of thymine dimers in a variety of aquatic organisms such as cyanobacteria, phytoplankton and macroalgae, a simple and efficient quantitative method was developed by Sinha et al., (2001) which is based on use of thymine dimer-specific antibodies followed by blotting and chemiluminescence methods. In this assay a plasmid pBSK with known DNA sequence, length and number of adjacent thymine pairs was used for calibration. By using the same method it was found that there was an increase from 3.2 to 50.9 thymine dimers per mega base pair during the light period in phytoplankton assemblage exposed at the surface under natural solar radiation from a freshwater lake in Trelew, Argentina (Klish et al., 2005).

DNA repair mechanism

Several specific repair enzymes and proteins are present in the organisms that continuously scan the genome for the occurrence of DNA lesions. Once a mismatched base, an apurinic or apyrimidinic site or structurally altered base is encountered, an efficient DNA repair is initiated, which in most cases leads to the restoration of the genetic information (Carell and Eppe, 1998; Sinha and Hider, 2002).

Photo-reactivation

Photo-reactivation or photo-enzymatic repair (PER) is one of the simplest and most important DNA repair system that involves the enzyme photolyase. The enzyme binds to CPD (CDP photolyase) or 6-4PPs (6-4 Photolyase) and reverses the damage using the energy of light. Long wavelength UV-A or blue light energy is absorbed by either 5,10-methylenetetrahydrofolate (MTHF) or 8-hydroxy-5-deazariboflavin (λmax ≈ 380 and 450 nm) and transferred to the catalytic cofactor, which is invariably a two-electron-reduced flavin adenine dimucleotide (FADH-). The whole process is known as photoreactivation (Fig. 1). The flavin in the excited state transfers an electron to the CDP, splitting the cyclobutane ring and the electron is transferred back to the flavin resulting in the generation of the two canonical bases (Sancar, 1994; Kim et al., 1994; Sancar, 1996; Thoma, 1999). Once the photolyase has been bound to a CDP, the efficiency of photoreactivation is extremely high with one dimer split for almost every blue light photon absorbed. The major photoreactivating factor, phrA, in the cyanobacterium Synechocystis sp. strain PCC 6803 codes for a CPD-specific DNA photolyase.

![Figure 1 Schematic representation of the pathway showing photoreactivation](image)

Base excision repair (BER)

BER is also important in repairing DNA damage by ionizing radiation and strong alkylating agents. DNA glycosylases are the key enzymes involved in BER which remove different types of modified bases by cleavage of the N-glycosidic bond between the base and the 2-deoxyribose moiety of the nucleotide residues. After the base is removed, the apurinic/apyrimidinic (AP) site is excised by an AP-endonuclease or an AP lyase, which nicks the DNA strand 5 or 3 to the AP site respectively followed by the excision of the remaining deoxyribose phosphate residue by a phosphodiesterase. Subsequently, the gap is filled by a DNA ligase (Fig. 2).
may be hyaline or coloured, smooth, homogeneous or lamellated. The species, in particular, possess pigmented sheaths due to the presence of a pigment, scytonemin. A characteristic feature of Scytonema sp. is the presence of false branches.

Culture medium

Table 1 shown the chemical composition of Chu 10 cultural medium.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Quantity (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO4.7H2O</td>
<td>0.025</td>
</tr>
<tr>
<td>CaCl2.2H2O</td>
<td>0.027</td>
</tr>
<tr>
<td>Na2S03.3H2O</td>
<td>0.044</td>
</tr>
<tr>
<td>Na2CO3</td>
<td>0.020</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>0.010</td>
</tr>
<tr>
<td>Fe-citrate</td>
<td>0.005</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.005</td>
</tr>
<tr>
<td>H3BO3</td>
<td>0.500</td>
</tr>
<tr>
<td>ZnSO4.7H2O</td>
<td>0.050</td>
</tr>
<tr>
<td>MnCl2.4H2O</td>
<td>0.500</td>
</tr>
<tr>
<td>CuSO4.5 H2O</td>
<td>0.020</td>
</tr>
<tr>
<td>Na2MoO4.2H2O</td>
<td>0.010</td>
</tr>
<tr>
<td>CoCl2</td>
<td>0.040</td>
</tr>
<tr>
<td>pH=8.0</td>
<td></td>
</tr>
</tbody>
</table>

Autoclaving

Solutions of inorganic sources i.e phosphate, Fe-citrate and citric acids were autoclaved separately and added to cool sterile medium. Organic chemicals and their analogues and other compounds were sterilized by filtration through Millipore membrane filters (0.22 μl) before use.

Isolation of DNA

The pellet obtained were washed twice with 1 ml of STE buffer (50 mM NaCl + 50 mM Tris-HCl, pH-8.0 + 5 mM EDTA) and re-suspended in a 500 μl TE (wash) buffer (50 mM Tris-HCl, pH-8.0 + 50 mM EDTA). Thereafter the cells were broken by sonification for 3 min on ice. Subsequently the cells were treated with 100 μg/ml of proteinase K. Thereafter 750 μl of prewarmed (55 °C) extraction buffer[3% (w/v) CTAB + 1% (w/v) sarkosyle + 20 mM EDTA + 1.4 M NaCl + 0.1 M TrisHCl, pH-8.0 + 1% (w/v) 2-mercaptoethanol] were added and incubated at 55 °C for one hour in a water bath with mixing by gentle inversion every 10 minute. The resulting suspension was allowed to cool for 1-2 minute and there after 750 ml Chloroform:isoamyl alcohol (24:1, v/v) were added and mixed by gentle inversion until an emulsion was formed. After centrifugation (10,000xg for 8 min at room temperature) the supernatant was transferred to sterile microcentrifuge tubes. Thereafter 2 volumes of ethanol and 0.1 volume of 3 M sodium acetate (pH-5.2) were added and kept at 0 °C overnight for the precipitation of DNA. Next day the precipitated DNA was centrifuged at 10,000 x g for 15 minute. The pellet obtained was briefly rinsed once with ice-cold 70 % ethanol, dried and re-hydrated with 30 ml TE buffer (10mM Tris-HCl, pH-8.0 + 1mM EDTA).

Purification of DNA

The purity of DNA was determined by calculating for ratio of O.D. at 260 and 280 (Table 2).

<table>
<thead>
<tr>
<th>Ratio between 260/280nm</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8-2.0</td>
<td>Pure DNA</td>
</tr>
<tr>
<td>&lt;1.8</td>
<td>Protein contamination</td>
</tr>
<tr>
<td>&gt;2.0</td>
<td>RNA contamination</td>
</tr>
</tbody>
</table>

**MATERIAL AND METHODS**

**Biological material**

The cyanobacterium Scytonema sp. was used for the present study that was routinely grown in Chu 10 (without N supplement) liquid medium at a temperature of 20°C and a white light illumination of 20 W/m². The organism belongs to the order Nostocales and family Scytonemataceae, having distinct morphological features. Scytonema sp. is filamentous and the filaments intertwine to form a scum of bluish green colour. Each filament, made up of spherical, oval or squarish cells, is surrounded by an individual mucilaginous sheath. The sheath...
DNA damaging agents

Rejoining of DSB

Homologous recombination

Figure 3 Diagrammatic view of double strand break (DSB) repair as a result of exposure of ionizing radiation, oxidative damage and spontaneous cleavage of the sugar phosphate backbone of the DNA molecule. A- Nonhomologous end joining and B- Homologous recombination. Both processes involves different multiprotein complex

Electrophoresis

5 μl of control as well as UV-treated DNA samples + 5 μl of BPB (1X) were mixed and added into the well in 0.8 % agarose gel (containing 0.5 μg/ml EtBr) immersed in electrophoresis buffer in a horizontal tank. Electrophoresis was run at 20 mA for the 1st 30 minute, than the current was increased to 120 mA for further 90-120 minute. After electrophoresis the gel was placed on a molecular imager system (Gel Doc, Bio-Rad) and photographs were taken for further analysis.

Immuo-dot-blot Assay

DNA samples were transferred to the membrane and washed once with TE buffer. The membrane was dried for one hour at 80 °C to immobilize the DNA. Subsequently, the membrane was incubated for 1 h in PBS-T with 5 % (w/v) skimmed milk powder to block the non-specific sites. Thereafter, the membrane was incubated with the primary antibody (anti-thymine dimer; diluted 1:10000 in PBS-T) for 2 h at room temperature and then washed (3 x 10 min) with PBS-T. Afterwards the membrane was incubated with the secondary antibody [(anti-mouse IgG (Fab specific) peroxdyase conjugate; diluted 1:10000 in PBS-T with 5 % skimmed milk powder) for 1h at room temperature and washed with PBS-T. Finally, the membrane was placed in a detection reagent for 1 min before scanning.

RESULTS AND DISCUSSION

Before assessing the impact of UV-B radiation on DNA, we observed its effects on growth and survival of the test organism Scytonema sp. It was observed that growth and survival were severely affected by UV-B radiation for different durations. DNA is one of the key targets for UV-B radiation in a number of organisms including cyanobacteria. The effect of UV-B radiation specifically on genomic DNA of the test organism was studied. Accordingly genomic DNA isolated from the exponentially grown culture was exposed to UV-B radiation for varying time periods and absorption spectra were determined at 260 nm both in control as well as UV-B irradiated DNA. It was found that there was a progressive increase in absorbance (at 260 nm) with increasing duration of UV-B treatment to DNA (data not shown). The increase in absorbance may be due to separation of two complementary strands of DNA (denaturation), a fact generally observed during the estimation of melting curve of DNA with temperature treatment. However, the denaturation of DNA caused by UV-B treatment seems irreversible since there was no decrease in absorbance of UV-B- exposed DNA following transfer to fluorescent light or in the dark. In this work it was observed that unirradiated cultures showed more distinct bands whereas DNA from cultures exposed to UV-B radiation showed loss of various fragments with increasing duration of UV-B (Fig. 4) radiation.

Figure 4 UV-B-induced damage of genomic DNA in the cyanobacterium Scytonema sp. (A) control, (B) 60 min, (C) 90 min, (D) 120 min, (E) 150 min, (F) 180 min

To detect the DNA lesions, equal amount of DNA from test organisms were loaded onto the nylon membrane. Figure 5 represents the blotting pattern of DNA from the cyanobacterium Scytonema sp. after increasing duration of UV radiation. There was a gradual increase in the integrity of the luminescence with increasing UV irradiation time.
Transmission of the genetic information from one cell to its daughter is must for survival of any organisms, which requires not only extreme accuracy in replication of DNA and precision in chromosome distribution, but also in the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations. Increase in the level of UV-B radiation is likely to induce changes in cyanobacterial community composition since there are great differences in the susceptibility of species to UV-induced damage. Species having the ability to accumulate UV screening substances or with more effective repair mechanisms will likely be favored.

CONCLUSION

UV-B induced DNA damage has also been reported in other cyanobacteria such as Anabaena sp, Nostoc sp, and in several other aquatic organisms (Kumar et al., 2004; Sinha et al., 2001). UV-B treatment causes loss in the cooperative binding property of DNA which is evident from the failure of complementary strands of DNA. Kumar et al., (2004), has observed UV-induced DNA damage by increase in absorbance of DNA following UV-B exposure and also by PCR assay. A number of methods are in use to determine the DNA damage in a variety of organisms (Sinha et al., 2001; Sommaruga and Buma, 2000; Pakker et al., 2000; Perdiz et al., 2000). UV-induced DNA degradation has been reported in the cyanobacterium Synechocystis by using radioactive methods and showing percentage radioactivity lost from DNA as a measure for DNA degradation. Buma et al., (1995) developed an immunofluorescent thymine dimer detection method by labeling dyes with antibody followed by a secondary antibody (Fluorescein isothiocyanate) staining and finally visualization of DNA damage with flow cytometry or fluorescence microscopy. Sinha et al. (2001) has presented a simple and efficient quantitative method to determine the frequency of thymine dimer in a variety of organisms in relatively short period of time by using blotting and chemiluminescence methods. This method permits the measurement of low as well as high levels of DNA lesions in nanogram quantities of DNA.

Acknowledgments: I am thankful to B.H.U, Department of Botany, India for providing lab facilities support. I am also grateful to Dr. R. P. Sinha, Professor in Department of Botany, Banaras Hindu University, India for their immense support, cooperation and valuable suggestions.

REFERENCES


